

Table 1: Cleavage of 75 human light chains.

	Enzyme	Recognition*	Nch	Ns	Planned location of site
5	AfeI	AGCgct	0	0	
	<b>AfIII</b>	<b>Cttaag</b>	0	0	<b>HC FR3</b>
	AgeI	Accgggt	0	0	
	<b>AscI</b>	<b>GGcgcgcc</b>	0	0	<b>After LC</b>
	BglII	Agatct	0	0	
10	BsiWI	Cgtacg	0	0	
	BspDI	ATcgat	0	0	
	BssHII	Gcgcg	0	0	
	BstBI	TTcgaa	0	0	
	DraIII	CACNNNgtg	0	0	
	EagI	Cggccg	0	0	
15	FseI	GGCCGGcc	0	0	
	FspI	TGCgca	0	0	
	HpaI	GTtaac	0	0	
	<b>MfeI</b>	<b>Caattg</b>	0	0	<b>HC FR1</b>
	MluI	Acgcgt	0	0	
20	NcoI	Ccatgg	0	0	<b>Heavy chain signal</b>
	NheI	Gctagc	0	0	<b>HC/anchor linker</b>
	NotI	GCggccgc	0	0	<b>In linker after HC</b>
	NruI	TCGcga	0	0	
	PacI	TTAAATtaa	0	0	
25	PmeI	GTAAaac	0	0	
	PmlI	CACgtg	0	0	
	PvuI	CGATcg	0	0	
	SacII	CCGCgg	0	0	
	SallI	Gtcgac	0	0	
30	<b>SfiI</b>	<b>GGCCNNNNnngcc</b>	0	0	<b>Heavy Chain signal</b>
	SgfI	GCGATcg	0	0	
	SnabI	TACgta	0	0	
	StuI	AGGcct	0	0	
	<b>XbaI</b>	<b>Tctaga</b>	0	0	<b>HC FR3</b>
35	AatII	GACGTC	1	1	
	AclI	AAcgtt	1	1	
	AseI	ATtaat	1	1	
	BsmI	GAATGCN	1	1	
40	BspEI	Tccgga	1	1	<b>HC FR1</b>
	<b>BstXI</b>	<b>CCANNNNNntgg</b>	1	1	<b>HC FR2</b>
	DrdI	GACNNNNnngtc	1	1	
	HindIII	Aagctt	1	1	
	PciI	Acatgt	1	1	
	SapI	gaagagc	1	1	
45	ScalI	AGTact	1	1	
	SexAI	Accwgg	1	1	
	SpeI	Actagt	1	1	
	TliI	Ctcgag	1	1	
	XbaI	Ctcgag	1	1	
50	BcgI	cgannnnnntgc	2	2	
	BlpI	GCtnagc	2	2	
	BssSI	Ctcgtg	2	2	
	<b>BstAPI</b>	<b>GCANNNNntgc</b>	2	2	
	EspI	GCtnagc	2	2	
	KasI	Ggcgc	2	2	
55	PflMI	CCANNNNntgg	2	2	
	XmnI	GAANNnnttc	2	2	

			3	3	LC signal seq
	ApaLI	Gtgcac	3	3	
	NaeI	GCCggc	3	3	
	NgoMI	Gccggc	3	3	
	PvuII	CAGctg	3	3	
5	RsrII	CGgwccg	3	3	
	BsrBI	GAGcgg	4	4	
	BsrDI	GCAATGNNn	4	4	
	BstZ17I	GTAtac	4	4	
10	EcoRI	Gaattc	4	4	
	Sphi	GCATGc	4	4	
	SspI	AATatt	4	4	
	AccI	GTmkaC	5	5	
	BclI	Tgatca	5	5	
15	BsmBI	Nnnnnngagacg	5	5	
	BsrGI	Tgtaca	5	5	
	DraI	TTTaaa	6	6	
	NdeI	CAtatg	6	6	HC FR4
	SwaI	ATTaaat	6	6	
20	BamHI	Ggatcc	7	7	
	Saci	GAGCTc	7	7	
	BciVI	GTATCCNNNNNN	8	8	
	BsaBI	GATNNnnatc	8	8	
	NsiI	ATGCAT	8	8	
25	Bsp120I	Gggccc	9	9	CH1
	Apai	GGGCCc	9	9	CH1
	PspOOMI	Gggccc	9	9	
	BspHI	Tcatga	9	11	
	EcoRV	GATatc	9	9	
30	AhdI	GACNNNNngtc	11	11	
	BbsI	GAAGAC	11	14	
	Psii	TTAtaa	12	12	
	BsaI	GGTCTCNnnnn	13	15	
	XmaI	Cccggg	13	14	
35	AvaI	Cycgrg	14	16	
	BglI	GCCNNNNnggc	14	17	
	AlwNI	CAGNNNctg	16	16	
	BspMI	ACCTGC	17	19	
	XcmI	CCANNNNNnnntgg	17	26	
40	BstEII	Ggttacc	19	22	HC FR4
	Sse8387I	CCTGCAgg	20	20	
	AvrII	Cctagg	22	22	
	HincII	GTYrac	22	22	
	BsgI	GTGCAG	27	29	
45	MscI	TGGcca	30	34	
	BseRI	NNnnnnnnnnctcc	32	35	
	Bsu36I	CCtnagg	35	37	
	PstI	CTGCAg	35	40	
	EcII	nnnnnnnnnnntccgcc	38	40	
50	PpuMI	RGgwccy	41	50	
	StyI	Ccwwgg	44	73	
	EcoO109I	RGgnccy	46	70	
	Acc65I	Ggtacc	50	51	
	KpnI	GGTACc	50	51	
55	BpmI	ctccag	53	82	
	AvaII	Ggwcc	71	124	

\* cleavage occurs in the top strand after the last upper-case base. For REs

that cut palindromic sequences, the lower strand is cut at the symmetrical site.

Table 2: Cleavage of 79 human heavy chains

	Enzyme	Recognition	Nch	Ns	Planned location of site
5	AfeI	AGCgct	0	0	
	AfIII	Cttaag	0	0	HC FR3
10	AscI	GGcgcgcc	0	0	After LC
	BsiWI	Cgtacg	0	0	
	BspDI	ATcgat	0	0	
15	BssHII	Gcgcbc	0	0	
	FseI	GGCCGGcc	0	0	
	HpaI	GTTaac	0	0	
20	NheI	Gctagc	0	0	HC Linker
	NotI	GCggccgc	0	0	In linker, HC/anchor
	NruI	TCGcga	0	0	
25	NsiI	ATGCAt	0	0	
	PacI	TTAAATTaa	0	0	
	PciI	Acatgt	0	0	
	PmeI	GTAAaaac	0	0	
30	PvuI	CGATcg	0	0	
	RsrII	CGgwccg	0	0	
	SapI	gaagagc	0	0	
35	SfiI	GGCCNNNNnngcc	0	0	HC signal seq
	SgfI	GCGATcgc	0	0	
40	SwaI	ATTTaaat	0	0	
	AclI	AAcgtt	1	1	
	AgeI	Accggt	1	1	
45	AseI	ATtaat	1	1	
	AvrII	Cctagg	1	1	
	BsmI	GAATGCN	1	1	
	BsrBI	GAGcgg	1	1	
50	BsrDI	GCAATGNnn	1	1	
	DraI	TTTaaa	1	1	
	FspI	TGCgca	1	1	
	HindIII	Aagctt	1	1	
	MfeI	Caattg	1	1	HC FR1
	NaeI	GCCggc	1	1	
55	NgoMI	Gccggc	1	1	
	SpeI	Actagt	1	1	
	Acc65I	Ggtacc	2	2	
	BstBI	TTcgaat	2	2	
	KpnI	GGTACc	2	2	
	MluI	Acgcgt	2	2	
	NcoI	Ccatgg	2	2	In HC signal seq
	NdeI	CAtatg	2	2	HC FR4
	PmlI	CACgtg	2	2	
60	XcmI	CCANNNNNnnnnntgg	2	2	
	BcgI	cgannnnnntgc	3	3	
	BclI	Tgatca	3	3	
	BglI	GCCNNNNnngc	3	3	
65	BsaBI	GATNNnnatc	3	3	
	BsrGI	Tgtaca	3	3	
	SnaBI	TACgta	3	3	
70	Sse8387I	CCTGCAGg	3	3	

	<b>ApaLI</b>	Gtgcac	4	4	LC Signal/FR1
	BspHI	Tcatga	4	4	
	BssSI	Ctcgtg	4	4	
	PsiI	TTAtaa	4	5	
5	Sphi	GCATGc	4	4	
	AhdI	GACNNNNnngtc	5	5	
	<b>BspEI</b>	<b>Tccgga</b>	<b>5</b>	<b>5</b>	<b>HC FR1</b>
	MscI	TGGcca	5	5	
	Saci	GAGCTc	5	5	
10	Scal	AGTact	5	5	
	SexAI	Accwgg	5	6	
	SspI	AATatt	5	5	
	TliI	Ctcgag	5	5	
	XbaI	Ctcgag	5	5	
15	BbsI	GAAGAC	7	8	
	BstAPI	GCANNNNNntgc	7	8	
	BstZ17I	GTAtac	7	7	
	EcoRV	GATattc	7	7	
20	EcoRI	Gaattc	8	8	
	BpI	GCtnagc	9	9	
	Bsu36I	CCtnagg	9	9	
	DraIII	CACNNNNtg	9	9	
	Espl	GCtnagc	9	9	
	StuI	AGGcc	9	13	
	XbaI	Tctaga	9	9	HC FR3
	<b>Bsp120I</b>	<b>Gggccc</b>	<b>10</b>	<b>11</b>	<b>CH1</b>
	<b>Apal</b>	<b>GGGCCc</b>	<b>10</b>	<b>11</b>	<b>CH1</b>
25	PspOOMI	Gggccc	10	11	
	BciVI	GTATCCNNNNNN	11	11	
30	Sall	Gtcgac	11	12	
	DrdI	GACNNNNnngtc	12	12	
	KasI	Ggcgcc	12	12	
	XmaI	Cccggg	12	14	
35	BglII	Agatct	14	14	
	HincII	GTYrac	16	18	
	BamHI	Ggatcc	17	17	
	PflMI	CCANNNNNntgg	17	18	
	BsmBI	Nnnnnnngagacg	18	21	
40	<b>BstXI</b>	<b>CCANNNNNntgg</b>	<b>18</b>	<b>19</b>	<b>HC FR2</b>
	XmnI	GAANNnnttc	18	18	
	SacII	CCGCgg	19	19	
	PstI	CTGCAG	20	24	
45	PvuII	CAGctg	20	22	
	AvaI	Cycgrg	21	24	
	EagI	Cggccc	21	22	
	AatII	GACGTC	22	22	
50	BspMI	ACCTGC	27	33	
	AccI	GTmkac	30	43	
	StyI	Ccwwgg	36	49	
	AlwNI	CAGNNNctg	38	44	
	BsaI	GGTCTCNnnnn	38	44	
55	PpuMI	RGgwccy	43	46	
	BsgI	GTGCAG	44	54	
	BseRI	NNnnnnnnnnctcctc	48	60	
	EciI	nnnnnnnnnntccgcc	52	57	
	<b>BstEII</b>	<b>Ggttnacc</b>	<b>54</b>	<b>61</b>	<b>HC Fr4, 47/79 have one</b>
	EcoO109I	RGgnccy	54	86	

BpmI ctccag  
AvaII Ggwcc

60 121  
71 140

Table 5 (continued): Use of *FokI* as "Universal Restriction Enzyme"

*FokI* - for dsDNA, | represents sites of cleavage

sites of cleavage  
5'-cacGGATGtg--nnnnnnnn|nnnnnnnn-3' (SEQ ID NO:15)  
3'-gtgCCTACac--nnnnnnnnnnn|nnn-5' (SEQ ID NO:16)  
RECOG  
NITION of *FokI*

Case I

5'---gtg|tatt-actgtgc..Substrate....-3' (SEQ ID NO:17)  
3'-cac-ataa|tgacacq-  
                  gtGTAGGcac\  
                  5'- caCATCCgtg/ (SEQ ID NO:18)

Case II

5'---gtgtatt|agac-tgc..Substrate....-3' (SEQ ID NO:19)  
              cacataa-tctg|acg-5'  
/gtgCCTACac  
\cacGGATGtg-3' (SEQ ID NO:20)

Case III (Case I rotated 180 degrees)

/gtgCCTACac-5'  
\cacGGATGtg-  
              gtgtctt|acag-tcc-3' Adapter (SEQ ID NO:21)  
3'---cacagaa-tgtc|agg..substrate....-5' (SEQ ID NO:22)

Case IV (Case II rotated 180 degrees)

3' - gtGTAGGcac\ (SEQ ID NO:23)  
 5' - gag|tctc-actqagc  
 Substrate 3' -...ctc-agag|tgactcg...-5' (SEQ ID NO:24)

### Improved *FokI* adapters

*FokI* - for dsDNA, | represents sites of cleavage

#### Case I

Stem 11, loop 5, stem 11, recognition 17

5' -...catgtg|tatt-actgtgc..Substrate....-3'  
 3' -gtacac-ataa|tgacac-  
 5' - caCATCCgtgc C  
 TTT

#### Case II

Stem 10, loop 5, stem 10, recognition 18

5' -...gtgtatt|agac-tgctgcc..Substrate....-3'  
 T TgtgCCTACac-  
 C cacGGATGtg-3'  
 TTT

#### Case III (Case I rotated 180 degrees)

Stem 11, loop 5, stem 11, recognition 20

T TgtgCCTACac-5'  
 G AcacGGATGtg-  
 TTT           gtgtctt|acag-tccattctg-3' Adapter  
 3' -...cacagaa-tgtc|aggtaagac..substrate....-5'

#### Case IV (Case II rotated 180 degrees)

Stem 11, loop 4, stem 11, recognition 17

3' - gtGTAGGcac T  
 5' - atcgag|tctc-actqagc  
 Substrate 3' -...tagctc-agag|tgactcg...-5'

**BseRI**

| sites of cleavage

5'-cacGAGGAGnnnnnnnnnn|nnnn-3'  
3'-gtgctcctcnnnnnnn|nnnnnn-5'  
RECOG  
NITION of *BseRI*

Stem 11, loop 5, stem 11, recognition 19

3'-.....gaacat|cg-ttaagccagta.....5'  
T-T<sub>1</sub> cttgta-gc|aattcggtcat-3'  
C GCTGAGGGAGTC-  
T cgactcctcag-5' An adapter for *BseRI* to cleave the substrate above.  
[T]

5'.....|cg-ttaagccagta.....5'  
T-T<sub>1</sub> cttgta-gc|aattcggtcat-3'  
C GCTGAGGGAGTC-  
T cgactcctcag-5'

Table 8: Matches to URE FR3 adapters in 79 human HC.

A. List of Heavy-chains genes sampled

	AF008566	af103343	HSA235676	HSU92452	HSZ93860
	AF035043	AF103367	HSA235675	HSU94412	HSZ93863
5	AF103026	AF103368	HSA235674	HSU94415	MCOMFRAA
	af103033	AF103369	HSA235673	HSU94416	MCOMFRVA
	AF103061	AF103370	HSA240559	HSU94417	S82745
	Af103072	af103371	HSCB201	HSU94418	S82764
	af103078	AF103372	HSIGGVH	HSU96389	S83240
10	AF103099	AF158381	HSU44791	HSU96391	SABVH369
	AF103102	E05213	HSU44793	HSU96392	SADEIGVH
	AF103103	E05886	HSU82771	HSU96395	SAH2IGVH
	AF103174	E05887	HSU82949	HSZ93849	SDA3IGVH
15	AF103186	HSA235661	HSU82950	HSZ93850	SIGVHTTD
15	af103187	HSA235664	HSU82952	HSZ93851	SUK4IGVH
	AF103195	HSA235660	HSU82961	HSZ93853	
	af103277	HSA235659	HSU86522	HSZ93855	
	af103286	HSA235678	HSU86523	HSZ93857	
	AF103309	HSA235677			

Table 8 B. Testing all distinct GLGs from bases 89.1 to 93.2 of the heavy variable domain

	Id	Nb	0	1	2	3	4	SEQ	ID NO:
	1	38	15	11	10	0	2	Seq1	gtgttattactgtgc
	2	19	7	6	4	2	0	Seq2	gtAtattactgtgc
	3	1	0	0	1	0	0	Seq3	gtgttattactgtAA
25	4	7	1	5	1	0	0	Seq4	gtgttattactgtAc
	5	0	0	0	0	0	0	Seq5	Ttgttattactgtgc
	6	0	0	0	0	0	0	Seq6	TtgttatCactgtgc
	7	3	1	0	1	1	0	Seq7	ACAtattactgtgc
	8	2	0	2	0	0	0	Seq8	ACgttattactgtgc
30	9	9	2	2	4	1	0	Seq9	ATgttattactgtgc
	Group		26	26	21	4	2		
	Cumulative		26	52	73	77	79		

Table 8C Most important URE recognition seqs in FR3 Heavy

1	VHSzy1	GTGtattactgtgc	(ON_SHC103)	(SEQ ID NO:25)
2	VHSzy2	GTAtattactgtgc	(ON_SHC323)	(SEQ ID NO:26)
3	VHSzy4	GTGtattactgtac	(ON_SHC349)	(SEQ ID NO:28)
5	4	VHSzy9	ATGtattactgtgc	(ON_SHC5a) (SEQ ID NO:33)

Table 8D, testing 79 human HC V genes with four probes

Number of sequences..... 79

Number of bases..... 29143

10

Id	Best	Number of mismatches					
		0	1	2	3	4	5
1	39	15	11	10	1	2	0
2	22	7	6	5	3	0	1
3	7	1	5	1	0	0	0
4	11	2	4	4	1	0	0
Group		25	26	20	5	2	
Cumulative		25	51	71	76	78	

20

One sequence has five mismatches with sequences 2, 4, and 9; it is scored as best for 2.

25

Id is the number of the adapter.

Best is the number of sequence for which the identified adapter was the best available.

The rest of the table shows how well the sequences match the adapters. For example, there are 11 sequences that match VHSzy1(Id=1) with 2 mismatches and are worse for all other adapters. In this sample, 90% come within 2 bases of one of the four adapters.

30 31 32 33 34 35 36 37 38 39 40 41 42 43

Table 130: PCR primers for amplification of human Ab genes

(HuIgMFOR) 5'-tgg aag agg cac gtt ctt ttc ttt-3'  
30 !(HuIgMFOREtop) 5'-aaa gaa aag aac gtg cct ctt cca-3' = reverse complement  
(HuCkFOR) 5'-aca ctc tcc cct gtt gaa gct ctt-3'  
(HuCL2FOR) 5'-tga aca ttc tgt agg ggc cac tg-3'  
(HuCL7FOR) 5'-aga gca ttc tgc agg ggc cac tg-3'  
! Kappa  
35 (CKForeAsc) 5'-acc gcc tcc acc ggg cgc gcc tta tta aca ctc tcc cct gtt-  
gaa gct ctt-3'  
(CL2ForeAsc) 5'-acc gcc tcc acc ggg cgc gcc tta tta tga aca ttc tgt-  
agg ggc cac tg-3'  
(CL7ForeAsc) 5'-acc gcc tcc acc ggg cgc gcc tta tta aga gca ttc tgc-  
40 agg ggc cac tg-3'

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Table 195: Human GLG FR3 sequences

45 ! VH1

! 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80

agg gtc acc atg acc agg gac acg tcc atc agc aca gcc tac atg  
! 81 82 82a 82b 82c 83 84 85 86 87 88 89 90 91 92  
gag ctg agc agg ctg aga tct gac gac acg gcc gtg tat tac tgt  
! 93 94 95

5       gcg aga ga ! 1-02# 1

aga gtc acc att acc agg gac aca tcc gcg agc aca gcc tac atg  
gag ctg agc agc ctg aga tct gaa gac acg gct gtg tat tac tgt  
gcg aga ga ! 1-03# 2

aga gtc acc atg acc agg aac acc tcc ata agc aca gcc tac atg  
10      gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt  
gcg aga gg ! 1-08# 3

aga gtc acc atg acc aca gac aca tcc acg agc aca gcc tac atg  
gag ctg agg agc ctg aga tct gac gac acg gcc gtg tat tac tgt  
gcg aga ga ! 1-18# 4

15      aga gtc acc atg acc gag gac aca tct aca gac aca gcc tac atg  
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt  
gca aca ga ! 1-24# 5

aga gtc acc att acc agg gac agg tct atg agc aca gcc tac atg  
gag ctg agc agc ctg aga tct gag gac aca gcc atg tat tac tgt  
gca aga ta ! 1-45# 6

aga gtc acc atg acc agg gac acg tcc acg agc aca gtc tac atg  
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt  
gcg aga ga ! 1-46# 7

aga gtc acc att acc agg gac atg tcc aca agc aca gcc tac atg  
gag ctg agc agc ctg aga tcc gag gac acg gcc gtg tat tac tgt  
gcg gca ga ! 1-58# 8

5 aga gtc acg att acc gcg gac gaa tcc acg agc aca gcc tac atg  
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt  
gcg aga ga ! 1-69# 9

aga gtc acg att acc gcg gac aaa tcc acg agc aca gcc tac atg  
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt  
gcg aga ga ! 1-e# 10

10 aga gtc acc ata acc gcg gac acg tct aca gac aca gcc tac atg  
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt  
gca aca ga ! 1-f# 11

! VH2

15 agg ctc acc atc acc aag gac acc tcc aaa aac cag gtg gtc ctt  
aca atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt  
gca cac aga c! 2-05# 12

agg ctc acc atc tcc aag gac acc tcc aaa agc cag gtg gtc ctt  
acc atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt  
gca cgg ata c! 2-26# 13

20 agg ctc acc atc tcc aag gac acc tcc aaa aac cag gtg gtc ctt  
aca atg acc aac atg gac cct gtg gac aca gcc acg tat tac tgt  
gca cgg ata c! 2-70# 14

! VH3

25 cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg  
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt  
gcg aga ga ! 3-07# 15

cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg  
caa atg aac agt ctg aga gct gag gac acg gcc ttg tat tac tgt  
gca aaa gat a! 3-09#16

30 cga ttc acc atc tcc agg gac aac gcc aag aac tca ctg tat ctg  
caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt  
gcg aga ga ! 3-11# 17

cga ttc acc atc tcc aga gaa aat gcc aag aac tcc ttg tat ctt  
caa atg aac agc ctg aga gcc ggg gac acg gct gtg tat tac tgt  
gca aga ga ! 3-13# 18

aga ttc acc atc tca aga gat gat tca aaa aac acg ctg tat ctg  
caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt  
acc aca ga ! 3-15# 19

cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg

caa atg aac agt ctg aga gcc gag gac acg gcc ttg tat cac tgt  
gcg aga ga ! 3-20# 20

cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg  
caa atg aac agc agt ctg aga gcc gag gac acg gct gtg tat tac tgt  
5       gcg aga ga ! 3-21# 21

cgg ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg  
caa atg aac agc agt ctg aga gcc gag gac acg gcc gta tat tac tgt  
gcg aaa ga ! 3-23# 22

cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg  
10      caa atg aac agc agt ctg aga gct gag gac acg gct gtg tat tac tgt  
gcg aaa ga ! 3-30# 23

cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg  
caa atg aac agc agt ctg aga gct gag gac acg gct gtg tat tac tgt  
gcg aga ga ! 3303# 24

15      cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg  
caa atg aac agc agt ctg aga gct gag gac acg gct gtg tat tac tgt  
gcg aaa ga ! 3305# 25

cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg  
caa atg aac agc agt ctg aga gcc gag gac acg gct gtg tat tac tgt  
gcg aga ga ! 3-33# 26

20      cga ttc acc atc tcc aga gac aac agc aaa aac tcc ctg tat ctg  
caa atg aac agt ctg aga act gag gac acc gcc ttg tat tac tgt  
gca aaa gat a! 3-43#27

cga ttc acc atc tcc aga gac aat gcc aag aac tca ctg tat ctg  
caa atg aac agc agt ctg aga gac gag gac acg gct gtg tat tac tgt  
gcg aga ga ! 3-48# 28

25      aga ttc acc atc tca aga gat ggt tcc aaa agc atc gcc tat ctg  
caa atg aac agc agt ctg aaa acc gag gac acg gca gcc gtg tat tac tgt  
act aga ga ! 3-49# 29

30      cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt  
caa atg aac agc agt ctg aga gcc gag gac acg gcc gtg tat tac tgt  
gcg aga ga ! 3-53# 30

aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt  
caa atg ggc agc agt ctg aga gct gag gac atg gct gtg tat tac tgt  
35      gcg aga ga ! 3-64# 31

aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt  
caa atg aac agc agt ctg aga gct gag gac acg gct gtg tat tac tgt  
gcg aga ga ! 3-66# 32

aga ttc acc atc tca aga gat gat tca aag aac tca ctg tat ctg

caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt  
gct aga ga ! 3-72# 33

agg ttc acc atc tcc aga gat gat tca aag aac acg gcg tat ctg  
caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt  
5 act aga ca ! 3-73# 34

cga ttc acc atc tcc aga gac aac gcc aag aac acg ctg tat ctg  
caa atg aac agt ctg aga gcc gag gac acg gct gtg tat tac tgt  
gca aga ga ! 3-74# 35

aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg cat ctt  
10 caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt  
aag aaa ga ! 3-d# 36

! VH4

cga gtc acc ata tca gta gac aag tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt  
15 gcg aga ga ! 4-04# 37

cga gtc acc atg tca gta gac acg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gcc gtg gac acg gcc gtg tat tac tgt  
gcg aga aa ! 4-28# 38

cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg  
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt  
20 gcg aga ga ! 4301# 39

cga gtc acc ata tca gta gac agg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt  
gct aga ga ! 4302# 40

cga gtt acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg act gcc gca gac acg gcc gtg tat tac tgt  
25 gcc aga ga ! 4304# 41

cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg  
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt  
gct aga ga ! 4-31# 42

cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gcc gcg gac acg gct gtg tat tac tgt  
30 gct aga ga ! 4-34# 43

cga gtc acc ata tcc gta gac acg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac tgt  
gct aga ca ! 4-39# 44

cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt  
35 gct aga ga ! 4-59# 45

cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt  
gcg aga ga ! 4-61# 46

5       cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg  
aag ctg agc tct gtg acc gcc gca gac acg gcc gtg tat tac tgt  
gcg aga ga ! 4-b# 47

! VH5

10      cag gtc acc atc tca gcc gac aag tcc atc agc acc gcc tac ctg  
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt  
gcg aga ca ! 5-51# 48

cac gtc acc atc tca gct gac aag tcc atc agc act gcc tac ctg  
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt  
gcg aga ! 5-a# 49

! VH6

15      cga ata acc atc aac cca gac aca tcc aag aac cag ttc tcc ctg  
cag ctg aac tct gtg act ccc gag gac acg gct gtg tat tac tgt  
gca aga ga ! 6-1# 50

! VH7

20      cggttt gtc ttc tcc ttg gac acc tct gtc agc acg gca tat ctg  
cag atc tgc agc cta aag gct gag gac act gcc gtg tat tac tgt  
gcg aga ga ! 74.1# 51

---

25

Table 250: REDaptors, Extenders, and Bridges used for Cleavage and Capture of Human Heavy Chains in FR3.

**A: HpyCH4V Probes of actual human HC genes**

!HpyCH4V in FR3 of human HC, bases 35-56; only those with TGca site

30 TGca;10,

RE recognition:tgca

of length 4 is expected at 10

1

6-1 agttctccctgcagctgaactc

2	3-11, 3-07, 3-21, 3-72, 3-48	cactgtatctgcaa atgaac ag
3	3-09, 3-43, 3-20	ccctgtatctgcaa atgaac ag
4		5-51 cgcctac ctgcag tggagg cag
5	3-15, 3-30, 3-30.5, 3-30.3, 3-74, 3-23, 3-33	cgctgtatctgcaa atgaac ag
6		7-4.1 cggcatatctgcag atctgcag
7		3-73 cggcgtatctgcaa atgaac ag
8		5-a ctgcctac ctgcag tggagg cag
9		3-49 tcgcctatctgcaa atgaac ag

10   **B: HpyCH4V REadaptors, Extenders, and Bridges**

**B.1 REadaptors**

! Cutting HC lower strand:

! TmKeller for 100 mM NaCl, zero formamide

! Edapters for cleavage

			T <sub>m</sub> <sup>w</sup>	T <sub>m</sub> <sup>k</sup>
15	(ON_HCFR36-1)	5'-agt tctcccTGCAGctgaactc-3'	68.0	64.5
	(ON_HCFR36-1A)	5'-tttctcccTGCAGctgaactc-3'	62.0	62.5
	(ON_HCFR36-1B)	5'-tttctcccTGCAGctgaac-3'	56.0	59.9
	(ON_HCFR33-15)	5'-cgctgtatcTGCAaatgaac ag-3'	64.0	60.8
	(ON_HCFR33-15A)	5'-ctgtatcTGCAaatgaac ag-3'	56.0	56.3
20	(ON_HCFR33-15B)	5'-ctgtatcTGCAaatgaac-3'	50.0	53.1
	(ON_HCFR33-11)	5'-cactgtatcTGCAaatgaac ag-3'	62.0	58.9
	(ON_HCFR35-51)	5'-ccgcctaccTGCAgtggagg cag-3'	74.0	70.1

!

**B.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned**

! XbaI...

! D323\* cgCttcacTaag tcT aqa gac aaC tcT aag aaT acT ctC taC  
! scab..... designed gene 3-23 gene.....

!

! HpyCH4V

! ... AflIII...

! Ttg caG atg aac agc TtA aqG . . .

! ..... . . .

**B.3 Extender and Bridges**

35   ! Extender (bottom strand):

!

(ON\_HCHpyEx01) 5' -cAAgTAgAgAgTATTcTTAgAgTTgTcTcTAqAcTTAgTgAAgcg-3'

! ON\_HCHpyEx01 is the reverse complement of

! 5'-cgCttcacTaag tcT aqa gac aaC tcT aag aaT acT ctC taC Ttg -3'

40   !

! Bridges (top strand, 9-base overlap):

!

(ON\_HCHpyBr016-1) 5'-cgCttcacTaag tcT aqa gac aaC tcT aag-  
aaT acT ctC taC Ttg CAgtgaac-3' {3'-term C is blocked}

!

5 ! 3-15 et al. + 3-11

(ON\_HCHpyBr023-15) 5'-cgCttcacTaag tcT aqa gac aaC tcT aag-  
aaT acT ctC taC Ttg CAaatgaac-3' {3'-term C is blocked}

!

! 5-51

10 (ON\_HCHpyBr045-51) 5'-cgCttcacTaag tcT aqa gac aaC tcT aag-  
aaT acT ctC taC Ttg CAgtggagc-3' {3'-term C is blocked}

!

! PCR primer (top strand)

!

15 (ON\_HCHpyPCR) 5'-cgCttcacTaag tcT aqa gac-3'

!

---

**C: BlpI Probes from human HC GLGs**

20	1	1-58,1-03,1-08,1-69,1-24,1-45,1-46,1-f,1-e	acatggaGCTGAGCaggctgag
	2		1-02 acatggaGCTGAGCaggctgag
	3		1-18 acatggagctgaggagcctgag
	4		5-51,5-a acctgcagtggagcagcctgaa
	5		3-15,3-73,3-49,3-72 atctgcaaataaacagcctgaa
25	6	3303,3-33,3-07,3-11,3-30,3-21,3-23,3305,3-48	atctgcaaataaacagcctgag
	7		3-20,3-74,3-09,3-43 atctgcaaataaacagtctgag
	8		74.1 atctgcagatctgcagccttaaa
	9		3-66,3-13,3-53,3-d atcttcaaataaacagcctgag
	10		3-64 atcttcaaataatgggcagcctgag
30	11	4301,4-28,4302,4-04,4304,4-31,4-34,4-39,4-59,4-61,4-b	ccctgaaGCTGAGCtctgtgac
	12		6-1 ccctgcagctgaactctgtgac
	13		2-70,2-05 tccttacaatgaccaacatgga
	14		2-26 tccttaccatgaccaacatgga

---

**D: BlpI REadaptors, Extenders, and Bridges**

35 D.1 REadaptors

		T <sub>m</sub> <sup>w</sup>	T <sub>m</sub> K
	(BlpF3HC1-58) 5'-ac atg gaG CTG AGC agc ctg ag-3'	70	66.4
	(BlpF3HC6-1) 5'-cc ctg aag ctg agc tct gtg ac-3'	70	66.4
40	! BlpF3HC6-1 matches 4-30.1, not 6-1.		

D.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

XbaI...  
D323\* cgCttcacTaag TCT AGA gac aaC tcT aag aaT acT ctc taC Ttg caG atg aac  
AflIII...  
agg CTTA AGG

### D.3 Extender and Bridges

! Bridges

**10** (BlpF3Br1) 5'-cgCttcacTcaag tcT aga gaT aaC AGT aaA aaT acT TtG-  
                  taC Ttg caG CtG a|GC aqc ctG-3'

(BlpF3Br2) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT Ttg-  
taC Ttq caG Ctq a|gc tct gta-3'

lower strand is cut here

15 ! Extender

(BlpF3Ext) 5' -

TcAgcTgcAAgTAcAAAAGTATTTTAcTgTTATcTcTAGAcTgAgTgAAgcg-3'

**! BlpF3Ext** is the reverse complement of:

! 5'-cgCttcacTcag tctT aga gaT aac AGT aaA aaT acT TtG tac Ttq caG Ctq a-3'

!

(BlpF3PCR) 5'-cgCttcacTcag tcT aga gaT aaC-3'

**E: HpyCH4III** Distinct GLG sequences surrounding site, bases 77-98

```

1 102#1,118#4,146#7,169#9,1e#10,311#17,353#30,404#37,4301 ccgtgtattactgtgcgagaga
2 103#2,307#15,321#21,3303#24,333#26,348#28,364#31,366#32 ctgtgtattactgtgcgagaga
3 108#3 ccgtgtattactgtgcgagagg
4 124#5,1f#11 ccgtgtattactgtgcacacaga
5 145#6 ccatgtattactgtgcacacaga
6 158#8 ccgtgtattactgtgcggcaga
7 205#12 ccacatattactgtgcacacag
8 226#13 ccacatattactgtgcacccgat
9 270#14 ccacgttattactgtgcacccgat
10 309#16,343#27 ccttgttattactgtgcacaaaga
11 313#18,374#35,61#50 ctgtgtattactgtgcacagaga
12 315#19 ccgtgtattactgttaccacacaga
13 320#20 ccttgttatcaactgtgcgagaga
14 323#22 ccgtatattactgtgcgaaaga
15 330#23,3305#25 ctgtgtattactgtgcgaaaga
16 349#29 ccgtgtattactgtactagaga
17 372#33 ccgtgtattactgtgcctagaga
18 373#34 ccgtgtattactgtactagacac
19 3d#36 ctgtgtattactgttaaaaaga
20 428#38 ccgtgtattactgtgcgagaaaa
21 4302#40,4304#41 ccgtgtattactgtgcacacaga
22 439#44 ctgtgtattactgtgcgagaca
23 551#48 ccatttattactgtgcgagaca

```

**F: HpyCH4III REadaptors, Extenders, and Bridges****F.1 REadaptors**

5	! ONs for cleavage of HC(lower) in FR3(bases 77-97)			
	! For cleavage with HpyCH4III, Bst4CI, or TaaI			
	! cleavage is in lower chain before base 88.			
	!			
10	(H43.77.97.1-02#1)	5'-cc gtg tat tAC TGT gcg aga g-3'	T <sub>m</sub> <sup>W</sup>	T <sub>m</sub> <sup>K</sup>
	(H43.77.97.1-03#2)	5'-ct gtg tat tAC TGT gcg aga g-3'	64	62.6
	(H43.77.97.108#3)	5'-cc gtg tat tAC TGT gcg aga g-3'	62	60.6
	(H43.77.97.323#22)	5'-cc gta tat tac tgt gcg aaa g-3'	64	62.6
	(H43.77.97.330#23)	5'-ct gtg tat tac tgt gcg aaa g-3'	60	58.7
15	(H43.77.97.439#44)	5'-ct gtg tat tac tgt gcg aga c-3'	60	58.7
	(H43.77.97.551#48)	5'-cc atg tat tac tgt gcg aga c-3'	62	60.6
	(H43.77.97.5a#49)	5'-cc atg tat tAC TGT gcg aga c-3'	58	58.3

**F.2 Extender and Bridges**

20	! XbaI and AflII sites in bridges are bunged			
	(H43.XABr1) 5'-ggtgttagtga-			
	TCT AGt gac aac tct aag aat act ctc tac ttg cag atg -			
	aac aqC TTt AGg gct qag qac aCT GCA Gtc tac tat tgt gcg aga-3'			
25	(H43.XABr2) 5'-ggtgttagtga-			
	TCT AGt gac aac tct aag aat act ctc tac ttg cag atg -			
	aac aqC TTt AGg gct qag qac aCT GCA Gtc tac tat tgt gcg aaa-3'			
	(H43.XAExt) 5'-ATAgTAgAcT gcAgTgTccT cAgcccTTAA gcTgTTcATc TgcAAgTAgA-			
	gAgTATTcTT AgAgTTgTcT cTAgATcAcT AcAcc-3'			
	!H43.XAExt is the reverse complement of			
30	! 5'-ggtgttagtga-			
	TCT AGA gac aac tct aag aat act ctc tac ttg cag atg -			
	aac aqC TTA AGg gct qag qac aCT GCA Gtc tac tat -3'			
	(H43.XAPCR) 5'-ggtgttagtga  TCT AGA gac aac-3'			
35	! XbaI and AflII sites in bridges are bunged			
	(H43.ABr1) 5'-ggtgttagtga-			
	aac aqC TTt AGg gct qag qac aCT GCA Gtc tac tat tgt gcg aga-3'			
	(H43.ABr2) 5'-ggtgttagtga-			
	aac aqC TTt AGg gct qag qac aCT GCA Gtc tac tat tgt gcg aaa-3'			
40	(H43.AExt) 5'-ATAgTAgAcTgcAgTgTccTcAgcccTTAAgTgTTcAcTAcAcc-3'			

```
! (H43.AExt) is the reverse complement of 5'-ggtgttagtga-
! |aac|agC|TTA|AGg|qct|qaq|qac|aCT|GCA|Gtc|tac|tat -3'
(H43.APCR)      5'-ggtgttagtga |aac|agC|TTA|AGg|qct|q-3'
```

印譜圖說卷之三

Table 510

Klact) 5'-cACATccGtg TtgcTT cAggATgtg-3'  
 VHEX881) 5'-AATAGTAAG TcgAGTgtc TAGccCTTA AgctgtttGAT cTgcaAGTAG-  
AgATATTCT TagATGTC TGAAGCTTA gtGAAGCG-3'  
 Note that VHEX881 is the reverse complement of the ON below

25

5' -ATCTGTT TTGTT TGATTG-3'

Klact)

25 30

5' -cacatccgtt TGT  
 5' -AATAGTAGAC TgcAGTgT  
 AgAGATTCT TAGAGTT  
 at VHEX881 is the rever-  
 [RC] 5' -cgCttcacTaag-  
 Scab . . . . .

Klact) Ex881)

25 30

5'-cgCTtcaCTaaG-  
 |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-  
 |aac|tagC|TTA|AGG|gct|gag|gac|act|GCA|Gtc|tac|tat|tgt|gct|agg-3'  
 5'-cgCTtcaCTaaG-  
 |VHBAA881) (VHBBB881)

open with a new in the new  
for each file

|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-  
|aac|tagC|TTA|AGG|gct|gag|gac|act|GCA|Gtc|tac|tat|tgt|Acg ag-3'  
(VH881PCR) 5'-cgCttcacTaag|TCT AGA|gac|aac -3'

Table 600: V3-23 VH framework with variated codons shown

! Sites to be varied--> \*\*\* \*\*\* \*\*\*  
 ! ---FR1--->|...CDR1.....|---FR2---  
 ! 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60  
 ! A S G F T F S S Y A M S W V R  
 ! |gct|TCC|GGA|ttc|act|ttc|tct|TCG|TAC|Gct|atg|tct|tgg|gtt|cgC| 143  
 ! |cgA|agg|cct|aag|tga|aag|aga|agc|atg|cga|tac|aga|acc|caa|gcg|  
 ! | BspEI | | BsiWI | | BstXI .  
 !  
 ! 10 Sites to be varies--> \*\*\* \*\*\* \*\*\*  
 ! ---FR2--->|...CDR2.....  
 ! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75  
 ! Q A P G K G L E W V S A I S G  
 ! |CAa|qct|ccT|GGt|aaa|ggt|ttg|qag|tgg|qtt|tct|gct|atc|tct|ggt| 188  
 ! |gtt|cgA|ggA|cca|ttt|cca|aac|ctc|acc|caa|aga|cgA|tag|aga|cca|  
 ! ...BstXI |  
 !  
 ! \*\*\* \*\*\*  
 ! ....CDR2.....|---FR3---  
 ! 20 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90  
 ! S G G S T Y Y A D S V K G R F  
 ! |tct|ggt|ggc|agt|act|tac|tat|qct|qac|tcc|qtt|aaa|ggt|cgc|ttc| 233  
 ! |aga|cca|ccg|tca|tga|atg|ata|cgA|ctg|agg|caa|ttt|cca|gco|aag|  
 !  
 ! -----FR3-----  
 ! 25 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105  
 ! T I S R D N S K N T L Y L Q M  
 ! |act|atc|TCT|AGA|gac|aac|tct|aat|act|ctc|tac|ttg|cag|atg| 278  
 ! |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|  
 ! | XbaI |  
 !  
 ! -----FR3----->|  
 ! 30 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120  
 ! N S L R A E D T A V Y Y C A K  
 ! |aac|agC|TTA|AGg|gct|qag|qac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa| 323  
 ! |ttg|tag|aat|tcc|cgA|ctc|ctg|tga|cgt|cag|atg|ata|acg|cgA|ttt|  
 ! |AflIII | | PstI |  
 !  
 ! .....CDR3.....|---FR4---  
 ! 35 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
 ! D Y E G T G Y A F D I W G Q G  
 ! |gac|tat|gaa|ggT|act|ggT|tat|qct|ttc|qAC|ATA|TGg|ggt|caa|ggt| 368  
 ! |ctg|ata|ctt|cca|tga|cca|ata|cgA|aag|ctg|tat|acc|cca|gtt|cca|  
 ! | NdeI |  
 !  
 ! -----FR4----->|  
 ! 40 136 137 138 139 140 141 142  
 ! T M V T V S S  
 ! |act|atG|GTC|ACC|gtc|tct|agt- 389  
 ! |tga|tac|cag|tgg|cag|aga|tca-  
 ! | BstEII |  
 !  
 ! 45 143 144 145 146 147 148 149 150 151 152  
 ! A S T K G P S V F P  
 ! gcc tcc acc aaG GGC CCa tcg GTC TTC ccc-3'  
 ! cgg agg tgg ttc ccc ggt aqc caq aag ggg-5' 419  
 ! Bsp120I. BbsI... (2/2)  
 ! ApaI....  
 !  
 ! 50 (SFPRMET) 5'-ctg tct gaa cG GCC cag ccG-3'  
 ! (TOPFR1A) 5'-ctg tct gaa cG GCC cag ccG GCC atg gcc-  
 ! gaa|gtt|CAA|TTG|tta|gag|tct|ggT|-  
 ! |ggc|ggT|ctt|gtt|cag|ctt|ggT|ggT|tct|tta-3'  
 ! |3'-caa|gtc|ggA|cca|cca|aga|aat|gca|gaa|aga|acg|cgA|-  
 ! |cgA|agg|cct|aag|tga|aag-5' ! bottom strand

```

(BOTFR2) 3'-acc|caa|gcg|-  

          |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|-5' ! bottom strand  

(BOTFR3) 3'- a|cga|ctg|agg|caa|ttt|cca|gcg|aag|-  

          |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|-  

          |ttg|tgc|aat|tcc|cga|ctc|ctg|tga-  

(F06)      5'-gC|TTA|AGG|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|-  

          |gac|tat|gaa|gg|act|gg|tat|gct|ttc|gaC|ATA|TGg|gg|t-c-3'  

(BOTFR4) 3'-cga|aag|ctg|tat|acc|cca|gtt|cca|-  

          |tga|tac|cag|tgg|cag|aga|tca-  

          egg agg tgg ttc ccc ggt agc cag aag ggg-5' ! bottom strand  

(BOTPRCPIM) 3'-gg ttc ccc ggt agc cag aag ggg-5'  

!  

! CDR1 diversity  

15 (ON-vgC1) 5'-|gct|TCC|GGA|ttc|act|tcc|tct|<1>|TAC|<1>|atq|<1>|-  

!                                         CDR1.....6859  

|tgg|gtt|cgC|CAA|gct|ccT|GG-3'  

!  

!<1> stands for an equimolar mix of {ADEFGHIKLMNPQRSTVWY}; no C  

20                                         (this is not a sequence)  

!  

! CDR2 diversity  

25 (ON-vgC2) 5'-gg|ttg|gag|tgg|gtt|tct|<2>|atc|<2>|<3>|-  

!                                         CDR2.....  

          |tct|gg|tgg|<1>|act|<1>|tat|gct|gac|tcc|gtt|aaa|gg-3'  

          CDR2.....  

!  

<1> is an equimolar mixture of {ADEFGHIKLMNPQRSTVWY}; no C  

<2> is an equimolar mixture of {YRWVGS}; no ACDEFHIKLMNPQT  

<3> is an equimolar mixture of {PS}; no ACDEFGHIKLMNQRTVWY

```

Table 800 (new)

The following list of enzymes was taken from  
<http://rebase.neb.com/cgi-bin/asymmlist>.

I have removed the enzymes that a) cut within the recognition, b)  
 cut on both sides of the recognition, or c) have fewer than 2  
 bases between recognition and closest cut site.

REBASE Enzymes  
 04/13/2001

Type II restriction enzymes with asymmetric recognition sequences:

Enzymes	Recognition Sequence	Isoschizomers	Suppliers
AarI	CACCTGCNNNN^NNNN	-	Y
AceIII	CAGCTCNNNNNNN^NNNN	-	-
Bbr7I	GAAGACNNNNNNN^NNNN	-	-
BbvI	GCAGCNNNNNNN^NNNN	-	Y
BbvII	GAAGACNN^NNNN		
Bce83I	CTTGAGNNNNNNNNNNNNNN_NN^	-	-
BceAI	ACGGCNNNNNNNNNNNN^NN	-	Y
BcefI	ACGGCNNNNNNNNNNNN^N	-	-
BciVI	GTATCCNNNN_N^	BfuI	Y
BfiI	ACTGGGNNNN_N^	BmrI	Y
BinI	GGATCNNNN^N		
BscAI	GCATCNNNN^NN	-	-
BseRI	GAGGAGNNNNNNNN_NN^	-	Y
BsmFI	GGGACNNNNNNNNNN^NNNN	BspLU11III	Y
BspMI	ACCTGCNNNN^NNNN	Acc36I	Y
EciI	GGCGGANNNNNNNN_NN^	-	Y
Eco57I	CTGAAGNNNNNNNNNNNNNN_NN^	BspKT5I	Y
FauI	CCCGCNNNN^NN	BstFZ438I	Y
FokI	GGATGNNNNNNNNN^NNNN	BstPZ418I	Y
GsuI	CTGGAGNNNNNNNNNNNNNN_NN^	-	Y
HgaI	GACGCNNNNN^NNNNN	-	Y
HphI	GGTGANNNNNNN_N^	AsuHPI	Y
MboII	GAAGANNNNNNN_N^	-	Y
MlyI	GAGTCNNNN^	SchI	Y
MmeI	TCCRACNNNNNNNNNNNNNNNN_NN^	-	-
Mn1I	CCTCNNNNNN_N^	-	Y
PleI	GAGTCNNNN^N	PpsI	Y
RleAI	CCCACANNNNNNNN_NNN^	-	-
SfaNI	GCATCNNNNN^NNNN	BspST5I	Y
SspD5I	GGTGANNNNNNN^	-	-
Sth132I	CCCGNNNN^NNNN	-	-
StsI	GGATGNNNNNNNNNN^NNNN	-	-
TaqII	GACCGANNNNNNNNNN_NN^	CACCCANNNNNNNN_NN^	-
Tth111II	CAARCANNNNNNNN_NN^	-	-
UbaPI	CGAACG	-	-

The notation is  $\wedge$  means cut the upper strand and  $\underline{\phantom{x}}$  means cut the lower strand. If the upper and lower strand are cut at the same place, then only  $\wedge$  appears.

Table 120: MALIA3, annotated

! MALIA3 9532 bases

!-----

1 aat gct act act att agt aga att gat gcc acc ttt tca gct cgc gcc  
 5 ! gene ii continued  
 49 cca aat gaa aat ata gct aaa cag gtt att gac cat ttg cga aat gta  
 97 tct aat ggt caa act aaa tct act cgt tcg cag aat tgg gaa tca act  
 145 gtt aca tgg aat gaa act tcc aga cac cgt act tta gtt gca tat tta  
 193 aaa cat gtt gag cta cag cac cag att cag caa tta agc tct aag cca  
 10 241 tcc gca aaa atg acc tct tat caa aag gag caa tta aag gta ctc tct  
 289 aat cct gac ctg ttg gag ttt gct tcc ggt ctg gtt cgc ttt gaa gct  
 337 cga att aaa acg cga tat ttg aag tct ttc ggg ctt cct ctt aat ctt  
 385 ttt gat gca atc cgc ttt gct tct gac tat aat agt cag ggt aaa gac  
 433 ctg att ttt gat tta tgg tca ttc tcg ttt tct gaa ctg ttt aaa gca  
 15 481 ttt gag ggg gat tca ATG aat att tat gac gat tcc gca gta ttg gac  
 ! RBS?..... Start gene x, ii continues  
 529 gct atc cag tct aaa cat ttt act att acc ccc tct ggc aaa act tct  
 577 ttt gca aaa gcc tct cgc tat ttt ggt ttt tat cgt cgt ctg gta aac  
 625 gag ggt tat gat agt gtt gct ctt act atg cct cgt aat tcc ttt tgg  
 673 cgt tat gta tct gca tta gtt gaa tgt ggt att cct aaa tct caa ctg  
 721 atg aat ctt tct acc tgt aat aat gtt gtt ccg tta gtt cgt ttt att  
 769 aac gta gat ttt tct tcc caa cgt cct gac tgg tat aat gag cca gtt  
 817 ctt aaa atc gca TAA  
 ! End X & II  
 25 832 ggtaattca ca  
 !  
 ! M1 E5 Q10 T15  
 843 ATG att aaa gtt gaa att aaa cca tct caa gcc caa ttt act act cgt  
 ! Start gene V  
 30 !  
 ! S17 S20 P25 E30  
 891 tct ggt gtt tct cgt cag ggc aag cct tat tca ctg aat gag cag ctt  
 !  
 ! V35 E40 V45  
 35 939 tgt tac gtt gat ttg ggt aat gaa tat ccg gtt ctt gtc aag att act  
 !  
 ! D50 A55 L60  
 987 ctt gat gaa ggt cag cca gcc tat gcg cct ggt cTG TAC Acc gtt cat  
 ! BsrGI...

! L65 V70 S75 R80  
1035 ctg tcc tct ttc aaa gtt ggt cag ttc ggt tcc ctt atg att gac cgt  
!  
! P85 K87 end of V  
5 1083 ctg cgc ctc gtt ccg gct aag TAA C  
!  
1108 ATG gag cag gtc gcg gat ttc gac aca att tat cag gcg atg  
Start gene VII  
!  
10 1150 ata caa atc tcc gtt gta ctt tgt ttc gcg ctt ggt ata atc  
!  
VII and IX overlap.  
! ..... S2 V3 L4 V5 S10  
1192 gct ggg ggt caa agA TGA gt gtt tta gtg tat tct ttc gcc tct ttc gtt  
15 ! End VII  
! |start IX  
! L13 W15 G20 T25 E29  
1242 tta ggt tgg tgc ctt cgt agt ggc att acg tat ttt acc cgt tta atg gaa  
!  
20 1293 act tcc tc  
!  
.... stop of IX, IX and VIII overlap by four bases  
1301 ATG aaa aag tct tta gtc ctc aaa gcc tct gta gcc gtt gct acc ctc  
Start signal sequence of viii.  
25 !  
1349 gtt ccg atg ctg tct ttc gct gct gag ggt gac gat ccc gca aaa gcg  
! mature VIII --->  
1397 gcc ttt aac tcc ctg caa gcc tca gcg acc gaa tat atc ggt tat gcg  
1445 tgg gcg atg gtt gtt gtc att  
30 1466 gtc ggc gca act atc ggt atc aag ctg ttt aag  
1499 aaa ttc acc tcg aaa gca ! 1515  
! ..... -35 ..  
!  
1517 agc tga taaaaccgat acaattaaag gctccttttg  
35 ! ..... -10 ...  
!  
1552 gagcctttt ttttGGAGAt ttt ! S.D. underlined  
!  
! <----- III signal sequence ----->

त्रिवेदी त्रिवेदी त्रिवेदी त्रिवेदी त्रिवेदी त्रिवेदी त्रिवेदी त्रिवेदी

M K K L L F A I P L V  
 1575 caac GTG aaa aaa tta tta ttc gca att cct tta gtt ! 1611  
 !  
 V P F Y S H S A Q  
 1612 gtt cct ttc tat tct cac aGT gcA Cag tCT  
 ! ApaLI...  
 !  
 1642 GTC GTG ACG CAG CCG CCC TCA GTG TCT GGG GCC CCA GGG CAG  
 AGG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC ATC GGG GCA  
 ! BstEII...  
 1729 GGT TAT GAT GTA CAC TGG TAC CAG CAG CTT CCA GGA ACA GCC CCC AAA  
 1777 CTC CTC ATC TAT GGT AAC AGC AAT CGG CCC TCA GGG GTC CCT GAC CGA  
 1825 TTC TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC ACT  
 1870 GGG CTC CAG GCT GAG GAT GAG GCT GAT TAT  
 1900 TAC TGC CAG TCC TAT GAC AGC AGC CTG AGT  
 1930 GGC CTT TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC  
 ! BstEII...  
 1969 CTA GGT CAG CCC AAG GCC AAC CCC ACT GTC ACT  
 2002 CTG TTC CCG CCC TCC TCT GAG GAG CTC CAA GCC AAC AAG GCC ACA CTA  
 2050 GTG TGT CTG ATC AGT GAC TTC TAC CCG GGA GCT GTG ACA GTG GCC TGG  
 2098 AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA CCC  
 2146 TCC AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAT CTG AGC CTG  
 2194 ACG CCT GAG CAG TGG AAG TCC CAC AGA AGC TAC AGC TGC CAG GTC ACC  
 2242 CAT GAA GGG AGC ACC GTG GAG AAG ACA GTG GCC CCT ACA GAA TGT TCA  
 2290 TAA TAA ACCG CCTCCACCGGCGCGCCAAT TCTATTCAA GGAGACAGTC ATA  
 ! AscI.....  
 !  
 ! PelB signal----->  
 !  
 M K Y L L P T A A A G L L L L L  
 2343 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC  
 !  
 16 17 18 19 20 21 22  
 ! A A Q P A M A  
 2388 gcG GCC cag ccG GCC atq gcc  
 ! SfiI.....  
 ! NgoMI... (1/2)  
 ! NcoI.....  
 !

! FR1 (DP47/V3-23) -----
 ! 23 24 25 26 27 28 29 30
 ! E V Q L L E S G
 ! 2409 gaa|gtt|CAA|TTG|ttt|gag|tct|ggg|
 ! | MfeI |
 !
 ! -----FR1-----
 !
 ! 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
 ! G G L V Q P G G S L R L S C A
 ! 2433 |ggc|ggg|ctt|gtt|cag|ctt|ggg|ttt|tta|cgt|ttt|tct|tg|gct|
 !
 ! -----FR1----->|...CDR1.....|---FR2-----
 !
 ! 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
 ! A S G F T F S S Y A M S W V R
 ! 2478 |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tg|gtt|cgC|
 ! | BspEI | | BsiWI | | BstXI |
 !
 ! -----FR2----->|...CDR2.....|
 !
 ! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
 ! Q A P G K G L E W V S A I S G
 ! 2523 |CAa|gct|ccT|GGt|aa|ggg|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggg|
 ! ...BstXI |
 !
 ! .....CDR2.....|---FR3---
 !
 ! 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
 ! S G G S T Y Y A D S V K G R F
 ! 2568 |tct|ggg|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aa|ggg|cg|ttc|
 !
 ! -----FR3-----
 !
 ! 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
 ! T I S R D N S K N T L Y L Q M
 ! 2613 |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
 ! | XbaI |
 !
 ! -----FR3----->|
 !
 ! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
 ! N S L R A E D T A V Y Y C A K
 ! 2658 |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tg|gct|aaa|

! |AflIII | PstI |
 !  
 ! .....CDR3.....|---FR4-----  
 ! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
 5 ! D Y E G T G Y A F D I W G Q G  
 2703 |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggT|caa|ggT|  
 ! | NdeI |(1/4) |
 !  
 ! -----FR4----->|
 10 ! 136 137 138 139 140 141 142  
 ! T M V T V S S  
 2748 |act|atG|GTC|ACC|gtc|tct|agt  
 ! | BstEII |
 ! From BstEII onwards, pV323 is same as pCES1, except as noted.  
 15 ! BstEII sites may occur in light chains; not likely to be unique in final  
 ! vector.  
 !  
 ! 143 144 145 146 147 148 149 150 151 152  
 ! A S T K G P S V F P  
 20 2769 gcc tcc acc aaG GGC CCa tcg GTC TTC ccc  
 ! Bsp120I. BbsI...(2/2)  
 ! ApaI....  
 !  
 ! 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167  
 25 ! L A P S S K S T S G G T A A L  
 2799 ctg gca ccC TCC TCc aag agc acc tct ggg ggc aca gcg gcc ctg  
 ! BseRI...(2/2)  
 !  
 ! 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182  
 30 ! G C L V K D Y F P E P V T V S  
 2844 ggc tgc ctg GTC AAG GAC TAC TTC CCC gaA CCG GTg acg gtg tcg  
 ! AgeI....  
 !  
 ! 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197  
 35 ! W N S G A L T S G V H T F P A  
 2889 tgg aac tca GGC GCC ctg acc agc ggc gtc cac acc ttc ccg gct  
 ! KasI...(1/4)

!  
 ! 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212

! V L Q S S G L Y S L S S V V T  
 2934 gtc cta cag tCt agc GGa ctc tac tcc ctc agc agc gta gtg acc  
 ! (Bsu36I...) (knocked out)  
 !  
 5 ! 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227  
 ! V P S S L G T Q T Y I C N V  
 2979 gtg ccC tCt tct agc tTG Ggc acc cag acc tac atc tgc aac gtg  
 ! (BstXI.....)N.B. destruction of BstXI & BpmI sites.  
 !  
 10 ! 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242  
 ! N H K P S N T K V D K K V E P  
 3024 aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc  
 !  
 ! 243 244 245  
 15 ! K S C A A A H H H H H H S A  
 3069 aaa tct tgt GCG GCC GCT cat cac cac cat cat cac tct gct  
 ! NotI.....  
 !  
 ! E Q K L I S E E D L N G A A  
 20 3111 gaa caa aaa ctc atc tca gaa gag gat ctg aat ggt gcc gca  
 !  
 !  
 ! D I N D D R M A S G A  
 3153 GAT ATC aac gat gat cgt atg gct AGC ggc gcc  
 25 ! rEK cleavage site..... NheI... KasI...  
 ! EcoRV..  
 !  
 ! Domain 1 -----  
 !  
 ! A E T V E S C L A  
 30 3183 gct gaa act gtt gaa agt tgt tta gca  
 !  
 !  
 ! K P H T E I S F  
 3210 aaa ccc cat aca gaa aat tca ttt  
 35 !  
 ! T N V W K D D K T  
 3234 aCT AAC GTC TGG AAA GAC GAC AAA ACT  
 !  
 ! L D R Y A N Y E G C L W N A T G V

3261 tta gat cgt tac gct aac tat gag ggt tgt ctg tgG AAT GCt aca ggc gtt  
! BsmI \_\_\_\_\_

! V V C T G D E T Q C Y G T W V P I

5 3312 gta gtt tgt act ggt GAC GAA ACT CAG TGT TAC GGT ACA TGG GTT cct att  
!

! G L A I P E N

3363 ggg ctt gct atc cct gaa aat  
!

10 ! L1 linker -----  
! E G G G S E G G G S  
3384 gag ggt ggt ggc tct gag ggt ggc ggt tct  
!  
! E G G G S E G G G T

15 3414 gag ggt ggc ggt tct gag ggt ggc ggt act  
!  
! Domain 2 -----  
3444 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat atc aac  
3495 cct ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct  
3546 aat cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag aat  
! BseRI \_\_\_\_\_

3597 aat agg ttc cga aat agg cag ggg gca tta act gtt tat acg ggc act  
3645 gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act cct  
3693 gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttC AGA  
20 ! AlwNI  
3741 GAC TGc gct ttc cat tct ggc ttt aat gaa gat cca ttc gtt tgt gaa  
! AlwNI  
3789 tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct  
!  
30 3834 ggc ggc ggc tct  
! start L2 -----  
3846 ggt ggt ggt tct  
3858 ggt ggc ggc tct  
3870 gag ggt ggt ggc tct gag ggt ggc ggt tct  
35 3900 gag ggt ggc ggc tct gag gga ggc ggt tcc  
3930 ggt ggt ggc tct ggt ! end L2  
!  
! Domain 3 -----  
! S G D F D Y E K M A N A N K G A

Protein sequence analysis

3945 tcc ggt gat ttt gat tat gaa aag aag atg gca aac gct aat aag ggg gct  
!  
! M T E N A D E N A L Q S D A K G  
3993 atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc  
5 !  
! K L D S V A T D Y G A A I D G F  
4041 aaa ctt gat tct gtc gct act gat tac ggt gct gct gct atc gat ggt ttc  
!  
! I G D V S G L A N G N G A T G D  
10 4089 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat  
!  
! F A G S N S Q M A Q V G D G D N  
4137 ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat  
!  
! S P L M N N F R Q Y L P S L P Q  
4185 tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct caa  
!  
! S V E C R P F V F S A G K P Y E  
4233 tcg gtt gaa tgt cgc cct ttt gtc ttt agc gct ggt aaa cca tat gaa  
20 !  
! F S I D C D K I N L F R  
4281 ttt tct att gat tgt gac aaa ata aac tta ttc cgt  
!  
! End Domain 3  
!  
! G V F A F L L Y V A T F M Y V F140  
4317 ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta ttt  
! start transmembrane segment  
!  
! S T F A N I L  
30 4365 tct acg ttt gct aac ata ctg  
!  
! R N K E S  
4386 cgt aat aag gag tct TAA ! stop of iii  
! Intracellular anchor.  
35 !  
! M1 P2 V L L5 G I P L L10 L R F L G15  
4404 tc ATG cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc ggt  
! Start VI  
!

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4451 ttc ctt ctg gta act ttg ttc ggc tat ctg ctt act ttt ctt aaa aag  
 4499 ggc ttc ggt aag ata gct att gct att tca ttg ttt ctt gct ctt att  
 4547 att ggg ctt aac tca att ctt gtg ggt tat ctc tct gat att agc gct  
 4595 caa tta ccc tct gac ttt gtt cag ggt gtt cag tta att ctc ccg tct  
 5 4643 aat gcg ctt ccc tgt ttt tat gtt att ctc tct gta aag gct gct att  
 4691 ttc att ttt gac gtt aaa caa aaa atc gtt tct tat ttg gat tgg gat  
 !  
 ! M1 A2 V3 F5 L10 G13  
 10 4739 aaa TAA t ATG gct gtt tat ttt gta act ggc aaa tta ggc tct gga  
 ! end VI Start gene I  
 !  
 ! 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28  
 ! K T L V S V G K I Q D K I V A  
 15 4785 aag acg ctc gtt agc gtt ggt aag att cag gat aaa att gta gct  
 !  
 ! 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43  
 ! G C K I A T N L D L R L Q N L  
 4830 ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa aac ctc  
 !  
 20 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58  
 ! P Q V G R F A K T P R V L R I  
 4875 ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga ata  
 !  
 ! 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73  
 ! P D K P S I S D L L A I G R G  
 25 4920 ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt  
 !  
 ! 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88  
 ! N D S Y D E N K N G L L V L D  
 30 4965 aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat  
 !  
 ! 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103  
 ! E C G T W F N T R S W N D K E  
 35 5010 gag tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa  
 !  
 ! 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118  
 ! R Q P I I D W F L H A R K L G  
 5055 aga cag ccg att att gat tgg ttt cta cat gct cgt aaa tta qga

! 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133  
! W D I I F L V Q D L S I V D K

5100 tgg gat att att ttt ctt gtt cag gac tta tct att gtt gat aaa

5 ! 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148  
! Q A R S A L A E H V V Y C R R

5145 cag gcg cgt tct gca tta gct gaa cat gtt gtt tat tgt cgt cgt

10 ! 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163  
! L D R I T L P F V G T L Y S L

5190 ctg gac aga att act tta cct ttt gtc ggt act tta tat tct ctt

15 ! 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178  
! I T G S K M P L P K L H V G V

5235 att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc gtt

20 ! 179 180 181 182 183 184 185 186 187 188 189 189 190 191 192 193  
! V K Y G D S Q L S P T V E R W

5280 gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg

25 ! 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208  
! L Y T G K N L Y N A Y D T K Q

5325 ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag

30 ! 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223  
! A F S S N Y D S G V Y S Y L T

5370 gct ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg

35 ! 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238  
! P Y L S H G R Y F K P L N L G

5415 cct tat tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt

40 ! 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253  
! Q K M K L T K I Y L K K F S R

5460 cag aag atg aaa tta act aaa ata tat ttg aaa aag ttt tct cgc

45 ! 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268  
! V L C L A I G F A S A F T Y S

5505 gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt aca tat agt

DNA sequence analysis

!

! 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283  
! Y I T Q P K P E V K K V V S Q  
5 5550 tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc tct cag  
!

! 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298  
! T Y D F D K F T I D S S Q R L  
5595 acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt ctt  
!

10 ! 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313  
! N L S Y R Y V F K D S K G K L  
5640 aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa TTA  
!

PacI

!

15 ! 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328  
! I N S D D L Q K Q G Y S L T Y  
5685 ATT AAt agc gac gat tta cag aag caa ggt tat tca ctc aca tat  
!

PacI

!

20 ! 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343  
! i I D L C T V S I K K G N S N E  
! iv  
5730 att gat tta tgt act gtt tcc att aaa aaa ggt aat tca aAT Gaa  
!

Start IV

!

25 ! 344 345 346 347 348 349  
! i I V K C N .End of I  
! iv L3 L N5 V I7 N F V10  
5775 att gtt aaa tgt aat TAA T TTT GTT

30 ! IV continued.....

5800 ttc ttg atg ttt gtt tca tca tct tct ttt gct cag gta att gaa atg  
5848 aat aat tcg cct ctg cgc gat ttt gta act tgg tat tca aag caa tca  
5896 ggc gaa tcc gtt att gtt tct ccc gat gta aaa ggt act gtt act gta  
5944 tat tca tct gac gtt aaa cct gaa aat cta cgc aat ttc ttt att tct  
35 5992 gtt tta cgt gct aat aat ttt gat atg gtt ggt tca att cct tcc ata  
6040 att cag aag tat aat cca aac aat cag gat tat att gat gaa ttg cca  
6088 tca tct gat aat cag gaa tat gat gat aat tcc gct cct tct ggt ggt  
6136 ttc ttt gtt ccg caa aat gat aat gtt act caa act ttt aaa att aat  
6184 aac gtt cgg gca aag gat tta ata cga gtt gtc gaa ttg ttt gta aag

6232 tct aat act tct aaa tcc tca aat gta tta tct att gac ggc tct aat  
6280 cta tta gtt gtt TCT gca cct aaa gat att tta gat aac ctt cct caa  
! ApaLI removed

5 6328 ttc ctt tct act gtt gat ttg cca act gac cag ata ttg att gag ggt  
6376 ttg ata ttt gag gtt cag caa ggt gat gct tta gat ttt tca ttt gct  
6424 gct ggc tct cag cgt ggc act gtt gca ggc ggt gtt aat act gac cgc  
6472 ctc acc tct gtt tta tct tct gct ggt tcg ttc ggt att ttt aat  
6520 ggc gat gtt tta ggg cta tca gtt cgc gca tta aag act aat agc cat  
6568 tca aaa ata ttg tct gtg cca cgt att ctt acg ctt tca ggt cag aag  
10 6616 ggt tct atc tct gtG GGC CAg aat gtc cct ttt att act ggt cgt gtg  
! MscI \_\_\_\_\_

15 6664 act ggt gaa tct gcc aat gta aat aat cca ttt cag acg att gag cgt  
6712 caa aat gta ggt att tcc atg agc gtt ttt cct gtt gca atg gct ggc  
6760 ggt aat att gtt ctg gat att acc agc aag gcc gat agt ttg agt tct  
6808 tct act cag gca agt gat gtt att act aat caa aga agt att gct aca  
6856 acg gtt aat ttg cgt gat gga cag act ctt tta ctc ggt ggc ctc act  
6904 gat tat aaa aac act tct caa gat tct ggc gta ccg ttc ctg tct aaa  
6952 atc cct tta atc ggc ctc ctg ttt agc tcc cgc tct gat tcc aac gag  
7000 gaa agc acg tta tac gtg ctc gtc aaa gca acc ata gta cgc gcc ctg  
20 7048 TAG cggcgatt  
! End IV

7060 aagcgcggcg ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca gcgccttagc  
7120 gccccgttcct ttccgtttct tcccttcctt tctcgccacg ttccGCCGGCt ttccccgtca  
! NgoMI \_\_\_\_\_

25 7180 agctctaaat cgggggctcc ctttagggtt ccgatttagt gctttacggc acctcgaccc  
7240 caaaaaactt gatttgggtg atggttCACG TAGTGggcca tegccctgat agacggttt  
! DraIII \_\_\_\_\_

30 7300 tcgccccttG ACGTTGGAGT Ccacgttctt taatagtggc ctcttgttcc aaactggAAC  
! DrdI \_\_\_\_\_

7360 aacactcaac cctatctcggt gctattcttt tgatttataa gggattttgc cgatttcgg  
7420 accaccatca aacaggattt tcgcctgctg gggcaaacca gcggtggaccc cttgctgcaa  
7480 ctctctcagg gccaggcggt gaaggccaat CAGCTGttgc cCGTCTCact ggtaaaaaga  
! PvuII. BsmBI.

35 7540 aaaaccaccc tGGATCC AAGCTT  
! BamHI HindIII (1/2)  
! Insert carrying bla gene

7563 gcaggtg gcactttcg gggaaatgtg cgccggaccc  
7600 ctatttgttt atttttctaa atacattcaa atatGTATCC gctcatgaga caataaccct  
! BciVI

7660 gataaatgct tcaataatat tgaaaaAGGA AGAgt  
! RBS.?...  
! Start bla gene  
7695 ATG agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg gca ttt  
5 7746 tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa gat gct  
7797 gaa gat cag ttg ggC gCA CGA Gtg ggt tac atc gaa ctg gat ctc aac agc  
! BssSI...  
! ApaLI removed  
7848 ggt aag atc ctt gag agt ttt cgc ccc gaa gaa cgt ttt cca atg atg agc  
10 7899 act ttt aaa gtt ctg cta tgt cat aca cta tta tcc cgt att gac gcc ggg  
7950 caa gaG CAA CTC GGT CGc cgg gcg tat tct cag aat gac ttg gtt gAG  
! BcgI \_\_\_\_\_ ScaI  
8001 TAC Tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa  
! ScaI\_  
15 8052 tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt  
8103 ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg cac aac atg  
! PvulI \_\_\_\_\_  
8154 ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc  
8205 ata cca aac gac gag cgt gac acc acg atg cct gta gca atg cca aca acg  
20 8256 tTG CGC Aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg caa caa  
! FspI....  
!  
8307 tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg cgc tcg  
8358 GCC ctt ccG GCt ggc tgg ttt att gct gat aaa tct gga gcc ggt gag cgt  
25 ! BglI \_\_\_\_\_  
8409 gGG TCT Cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc tcc cgt  
! BsaI \_\_\_\_\_  
8460 atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa cga aat  
! AhdI \_\_\_\_\_  
30 8511 aga cag atc gct gag ata ggt gcc tca ctg att aag cat tgg TAA ctgt  
! stop  
8560 cagaccaagt ttactcatat atactttaga ttgattaaa acttcattt taattaaaa  
8620 ggatctagggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagttt  
8680 cgttccactg tacgtaaagac cccc  
35 8704 AAGCTT GTCGAC tgaa tggcgaatgg cgctttgcct  
! HindIII SalI..  
! (2/2) HincII  
8740 ggttccggc accagaagcg gtgccggaaa gctggctgga gtgcgatctt  
!

8790 CCTGAGG  
! Bsu36I\_  
8797 ccgat actgtcgctcg tcccctcaaa ctggcagatg  
8832 cacggttacg atgcgccat ctacaccaac gtaacctatac ccattacggt caatccgccc  
8892 tttgttcca cgagaatcc gacgggttgt tactcgctca catttaatgt tgatgaaagc  
8952 tggctacagg aaggccagac gcgaattattt tttgatggcg ttccatttgg taaaaaaatg  
9012 agctgattta acaaaaattt aacgcgaattt ttaacaaaat attaacgttt acaATTAAA  
!  
9072 Tatttgctta tacaatcttc ctgttttgg ggctttctg attatcaacc GGGGTACat  
10 ! RBS?  
9131 ATG att gac atg cta gtt tta cga tta ccg ttc atc gat tct ctt gtt tgc  
! Start gene II  
9182 tcc aga ctc tca ggc aat gac ctg ata gcc ttt gtA GAT CTc tca aaa ata  
!  
15 9233 gct acc ctc tcc ggc atg aat tta tca gct aga acg gtt gaa tat cat att  
9284 gat ggt gat ttg act gtc tcc ggc ctt tct cac cct ttt gaa tct tta cct  
9335 aca cat tac tca ggc att gca ttt aaa ata tat gag ggt tct aaa aat ttt  
9386 tat cct tgc gtt gaa ata aag gct tct ccc gca aaa gta tta cag ggt cat  
9437 aat gtt ttt ggt aca acc gat tta gct tta tgc tct gag gct tta ttg ctt  
20 9488 aat ttt gct aat tct ttg cct tgc ctg tat gat tta ttg gat gtt ! 9532  
! gene II continues

Table 120B: Sequence of MALIA3, condensed

LOCUS	MALIA3	9532	CIRCULAR
ORIGIN			
5	1	AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTCA G CTCGC GCCCC AAATGAAA AT	
	61	ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA ATGGTCAAAC TAAATCTACT	
	121	CGTTCGCAGA ATTGGGAATC AACTGTTACA TGGAATGAAA CTTCCAGACA CCGTACTTTA	
	181	GTTGCATATT TAAAACATGT TGAGCTACAG CACCAGATTG AGCAATTAAG QTCTAAGCCA	
	241	TCCGCAAAAA TGACCTTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG	
	301	TTGGAGTTTG CTTCCGGTCT GGTTCGCTT GAAGCTCGAA TTAAAACGCG ATATTTGAAG	
10	361	TCTTTCGGGC TTCCTCTTAA TCTTTTGAT GCAATCCGCT TTGCTTCTGA CTATAATAGT	
	421	CAGGGTAAAG ACCTGATTT TGATTTATGG TCATTCTCGT TTTCTGAAC GTTAAAGCA	
	481	TTTGAGGGGG ATTCAATGAA TATTTATGAC GATTCCCAG TATTGGACGC TATCCAGTCT	
	541	AAACATTTA CTATTACCCC CTCTGGCAA ACTTCTTTG CAAAAGCCTC TCGCTATTTT	
	601	GGTTTTTATC GT CGTCTGGT AAACGAGGGT TATGATAGT TTGCTCTTAC TATGCCTCGT	
15	661	AATTCCCTTT GGC GTTATGT ATCTGCATTA GTTGAATGTG GTATTCCTAA ATCTCAACTG	
	721	ATGAATCTT CTACCTGTAA TAATGTTGTT CCGTTAGTTC GTTTTATTAA CGTAGATTTT	
	781	TCTTCCCAAC GT CCTGACTG GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA	
	841	CAATGATTAA AGTTGAAATT AAACCATCTC AAGCCCAATT TACTACTCGT TCTGGTGT	
	901	CTCGTCAGGG CAAGCCTTAT TCACTGAATG AGCAGCTTG TTACGTTGAT TTGGGTAATG	
	961	AATATCCGGT TCTTGTCAAG ATTACTCTTG ATGAAGGTCA GCCAGCCTAT GCGCCTGGTC	
20	1021	TGTACACCGT TCATCTGTCC TCTTCAAAG TTGGTCAGTT CGGTCCCTT ATGATTGACC	
	1081	GTCTGCGCCT CGTTCCGGCT AAGTAACATG GAGCAGGTG CGGATTCGA CACAATTAT	
	1141	CAGGCGATGA TACAAATCTC CGTTGTACTT TGTTCGCGC TTGGTATAAT CGCTGGGGT	
	1201	CAAAGATGAG TGT TTTAGTG TATTCTTCG CCTCTTCGT TTTAGGTTGG TGCCCTCGTA	
	1261	GTGGCATTAC GTATTTTAC CGTTTAATGG AAACCTCCTC ATGAAAAAGT CTTTAGTCCT	
	1321	CAAAGCCTCT GTAGCCGTTG CTACCCCTCGT TCCGATGCTG TCTTCGCTG CTGAGGGTGA	
	1381	CGATCCCGCA AAAGCGGCCT TTAACCTCCCT GCAAGCCTCA GCGACCGAAT ATATCGGTTA	
	1441	TGCGTGGCG ATGGTTGTTG TCATTGTCGG CGCAACTATC GGTATCAAGC TGTTAAGAA	
	1501	ATTCACCTCG AAAGCAAGCT GATAAACCGA TACAATTAAA GGCTCCTTT GGAGCCTTT	
30	1561	TTTTGGAGA TTTCAACGT GAAAAAATTA TTATTCGCAA TTCTTTAGT TGTTCCCTTC	
	1621	TATTCTCACA GTGCACAGTC TGCGTGACG CAGCCGCCCT CAGTGTCTGG GGCCCCAGGG	
	1681	CAGAGGGTCA CCATCTCTG CACTGGGAGC AGCTCCAACA TCGGGGCAGG TTATGATGTA	
	1741	CACTGGTACC AGCAGCTTCC AGAACACAGCC CCCAAACTCC TCATCTATGG TAACAGCAAT	
	1801	CGGCCCTCAG GGGTCCCTGA CCGATTCTCT GGCTCCAAGT CTGGCACCTC AGCCTCCCTG	
35	1861	GCCATCACTG GGCTCCAGGC TGAGGGATGAG GCTGATTACT ACTGCCAGTC CTATGACAGC	
	1921	AGCCTGAGTG GCCTTATGT CTTCGGAAC TGGACCAAGG TCACCGTCCT AGGTCAGCCC	
	1981	AAGGCCAACCC CCACTGTCAC TCTGTTCCCG CCCTCCTCTG AGGAGCTCCA AGCCAACAAG	
	2041	GCCACACTAG TGTGTCTGAT CAGTGACTTC TACCCGGGAG CTGTGACAGT GGCTGGAAAG	
	2101	GCAGATAGCA GCCCGTCAA GGCGGGAGTG GAGACCACCA CACCCCTCAA ACAAGCAAC	

2161 ACAAGTACG CGGCCAGCAG CTATCTGAGC CTGACGCCCTG AGCAGTGGAA GTCCCACAGA  
2221 AGCTACAGCT GCCAGGTCACT GCATGAAGGG AGCACCGTGG AGAAGACAGT GGCCCCCTACA  
2281 GAATGTTCAT AATAAACCGC CTCCACCGGG CGCGCCAATT CTATTCAAG GAGACAGTCA  
2341 TAATGAAATA CCTATTGCCT ACGGCAGCCG CTGGATTGTT ATTACTCGCG GCCCAGCCGG  
5 2401 CCATGGCCGA AGTTCAATTG TTAGAGTCTG GTGGCGGTCT TGTCAGCCT GGTGGTTCTT  
2461 TACGTCTTTC TTGCGCTGCT TCCGGATTCA CTTTCTCTTC GTACGCTATG TCTTGGGTTCTC  
2521 GCCAAGCTCC TGGTAAAGGT TTGGAGTGGG TTTCTGCTAT CTCTGGTTCT GGTGGCAGTA  
2581 CTTACTATGC TGACTCCGTT AAAGGTCGCT TCACTATCTC TAGAGACAAC TCTAAGAATA  
2641 CTCTCTACTT GCAGATGAAC AGCTTAAGGG CTGAGGACAC TGAGTCTAC TATTGCGCTA  
10 2701 AAGACTATGA AGGTACTGGT TATGCTTCG ACATATGGGG TCAAGGTACT ATGGTCACCG  
2761 TCTCTAGTGC CTCCACCAAG GGCCCACATCGG TCTTCCCCCT GGCAACCTCC TCCAAGAGCA  
2821 CCTCTGGGGG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA CTACTTCCCC GAACCGGTGA  
2881 CGGTGTCGTG GAACTCAGGC GCCCTGACCA GCGGCGTCCA CACCTTCCCG GCTGTCCTAC  
2941 AGTCTAGCGG ACTCTACTCC CTCAGCAGCG TAGTGACCCT GCCCTCTTCT AGCTTGGCA  
15 3001 CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCCAGCAA CACCAAGGTG GACAAGAAAAG  
3061 TTGAGCCAA ATCTTGTGCG GCCGCTCATC ACCACCATCA TCACCTCTGCT GAACAAAAAC  
3121 TCATCTCAGA AGAGGATCTG AATGGTGCCTG CAGATATCAA CGATGATCGT ATGGCTGGCG  
3181 CCGCTGAAAC TGTGAAAGT TGTGAAAGT AACCCCATAC AGAAAATTCA TTTACTAACG  
3241 TCTGGAAAGA CGACAAAAGT TTAGATCGTT ACGCTAACTA TGAGGGTTGT CTGTGGAATG  
3301 CTACAGGCCTG TGTAGTTGT ACTGGTGACG AAACTCAGTG TTACGGTACA TGGGTTCTA  
3361 TTGGGCTTGC TATCCCTGAA AATGAGGGTG GTGGCTCTGA GGGTGGCGGT TCTGAGGGTG  
3421 GCGGTTCTGA GGGTGGCGGT ACTAAACCTC CTGAGTACGG TGATACACCT ATTCCGGCT  
3481 ATACTTATAT CAACCCCTCTC GACGGCACTT ATCCGCTGG TACTGAGCAA AACCCCGCTA  
3541 ATCCAATCC TTCTCTTGAG GAGTCTCAGC CTCTTAATAC TTTATGTTT CAGAATAATA  
3601 GGTTCCGAA TAGGCAGGGG GCATTAACTG TTTATACGGG CACTGTTACT CAAGGCACCTG  
3661 ACCCCGTTAA AACTTATTAC CAGTACACTC CTGTATCATC AAAAGCCATG TATGACGCTT  
3721 ACTGGAACGG TAAATTCTAGA GACTGCGCTT TCCATTCTGG CTTTAATGAA GATCCATTCTG  
3781 TTTGTGAATA TCAAGGCCAA TCGTCTGACC TGCCCTAACCC TCCCTGCAAT GCTGGCGCG  
3841 GCTCTGGTGG TGTTCTGGT GGCAGGCTCTG AGGGTGGTGG CTCTGAGGGT GGCAGGTTCTG  
3901 AGGGTGGCGG CTCTGAGGGG GGCAGGTTCCG GTGGTGGCTC TGGTCCGGT GATTTTGATT  
3961 ATGAAAAGAT GGCAACAGCT AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC  
4021 TACAGTCTGA CGCTAAAGGC AAACCTTGATT CTGTCGCTAC TGATTACGGT GCTGCTATCG  
4081 ATGGTTTCAT TGGTGACGTT TCCGGCCTTG CTAATGGTAA TGGTGCTACT GGTGATTTG  
4141 CTGGCTCTAA TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTACACCT TTAATGAATA  
35 4201 ATTTCCGTCA ATATTACCT TCCCTCCCTC AATCGGTTGA ATGTCGCCCT TTTGTCTTTA  
4261 GCGCTGGTAA ACCATATGAA TTTCTATTG ATTGTGACAA AATAAACTTA TTCCGTGGTG  
4321 TCTTTGCGTT TCTTTATAT GTGCCACCT TTATGTATGT ATTTCTACG TTTGCTAACAC  
4381 TACTGCGTAA TAAGGAGTCT TAATCATGCC AGTTCTTTG GGTATTCCGT TATTATTGCG  
4441 TTTCCTCGGT TTCCCTCTGG TAACTTTGTT CGGCTATCTG CTTACTTTTC TAAAAAAGGG

4501 CTTCGGTAAG ATAGCTATTG CTATTCATT GTTTCTGCT CTTATTATTG GGCTTAAC  
4561 AATTCTTGTG GGTTATCTCT CTGATATTAG CGCTCAATTA CCCTCTGACT TTGTTCA  
4621 TGTTCAGTTA ATTCTCCCGT CTAATGCGCT TCCCTGTTTT TATGTTATTCT TCTCTGTA  
4681 GGCTGCTATT TTCATTTTG ACAGTTAAACA AAAAATCGTT TCTTATTGATTGGATA  
5 4741 ATAATATGGC TGTTTATTGTT GTAACGGCA AATTAGGCTC TGGAAAGACG CTCGTTAGCG  
4801 TTGGTAAGAT TCAGGATAAA ATTGTAGCTG GGTGAAAAT AGCAACTAAT CTTGATTAA  
4861 GGCTTCAAAA CCTCCCGCAA GTCGGGAGGT TCGCTAAAAC GCCTCGCGTT CTTAGAATAC  
4921 CGGATAAGCC TTCTATATCT GATTGCTTG CTATTGGCG CGGTAAATGAT TCCTACGATG  
4981 AAAATAAAAAA CGGCTTGCTT GTTCTCGATG AGTGCAGTAC TTGGTTTAAT ACCCGTTCTT  
10 5041 GGAATGATAA GGAAAGACAG CCGATTATTG ATTGGTTCT ACATGCTCGT AAATTAGGAT  
5101 GGGATATTAT TTTTCTGTT CAGGACTTAT CTATTGTTGA TAAACAGGCG CGTTCTGCAT  
5161 TAGCTGAACA TGTTGTTAT TGTGTCGTC TGGACAGAAT TACCTTACCT TTTGTCGTTA  
5221 CTTTATATTCT TCTTATTACT GGCTCGAAAA TGCCCTGCC TAAATTACAT GTTGGCGTTG  
5281 TTAAATATGG CGATTCTCAA TTAAGCCCTA CTGTTGAGCG TTGGCTTTAT ACTGGTAAGA  
15 5341 ATTTGTATAA CGCATATGAT ACTAACACGG CTTTTCTAG TAATTATGAT TCCGGTGT  
5401 ATTCTTATTAA AACGCCTTAT TTATCACACG GTGGTATT CAAACCATTAA AATTAGGTC  
5461 AGAAGATGAA ATTAACAAA ATATATTGA AAAAGTTTC TCGCGTTCTT TGTCTTGC  
5521 TTGGATTGTC ATCAGCATT ACATATAGTT ATATAACCCA ACCTAACCG GAGGTT  
5581 AGGTAGTCTC TCAGACCTAT GATTTGATA AATTCACTAT TGACTCTTCT CAGCGTCTT  
20 5641 ATCTAAGCTA TCGCTATGTT TTCAAGGATT CTAAGGGAAA ATTAATTAAT AGCGACGATT  
5701 TACAGAAGCA AGGTTATTCA CTCACATATA TTGATTATG TACTGTTCC ATTAAAAAAG  
5761 GTAATTCAA TGAAATTGTT AAATGTAATT AATTTGTTT TCTTGATGTT TGTTTCATCA  
5821 TCTTCTTTG CTCAGGTAAT TGAAATGAAT AATTGCCCTC TGCGCGATT TGTAAC  
5881 TATTCAAAGC AATCAGGCGA ATCCGTTATT GTTCTCCCG ATGAAAAGG TACTGTTACT  
25 5941 GTATATTCACT CGACGTTAA ACCTGAAAAT CTACGCAATT TCTTATTCT TGTTTACGT  
6001 GCTAATAATT TTGATATGGT TGTTCAATT CCTTCATAA TTCAAGGTA TAATCCAAAC  
6061 AATCAGGATT ATATTGATGA ATTGCCATCA TCTGATAATC AGGAATATGA TGATAATT  
6121 GCTCCTTCTG GTGGTTCTT TGTTCCGCAA AATGATAATG TTACTCAAAC TTTTAA  
6181 AATAACGTTC GGGCAAAGGA TTTAATACGA GTTGTGAAT TGTTGTAAA GTCTAATACT  
30 6241 TCTAAATCCT CAAATGTATT ATCTATTGAC GGCTCTAAC TATTAGTTGT TTCTGCACCT  
6301 AAAGATATTAA TAGATAACCT TCCTCAATT CTTCTACTG TTGATTGCCC AACTGAC  
6361 ATATTGATTG AGGGTTGAT ATTTGAGGTT CAGCAAGGTG ATGTTTAGA TTTTTCTT  
6421 GCTGCTGGCT CTCAGCGTGG CACTGTTGCA GGCAGGTGTTA ATACTGACCG CCTCAC  
6481 GTTTTATCTT CTGCTGGTGG TTCGTTCGGT ATTTTAATG GCGATGTTT AGGGCTATCA  
35 6541 GTTCGCGCAT TAAAGACTAA TAGCCATTCA AAAATATTGT CTGTGCCACG TATTCTTAC  
6601 CTTTCAGGTC AGAAGGGTTC TATCTCTGTT GGCCAGAATG TCCCTTTAT TACTGGCGT  
6661 GTGACTGGTG AATCTGCCAA TGAAATAAT CCATTCAAGA CGATTGAGCG TCAAAATGTA  
6721 GGTATTTCGA TGAGCGTTT TCCGTGCA ATGGCTGGCG GTAATATTGT TCTGGATATT  
6781 ACCAGCAAGG CCGATAGTTT GAGTTCTTCT ACTCAGGCAA GTGATGTTAT TACTAATCAA

6841 AGAAGTATTG CTACAACGGT TAATTTGCGT GATGGACAGA CTCTTTACT CGGTGGCCTC  
 6901 ACTGATTATA AAAACACTTC TCAAGATTCT GGCGTACCGT TCCTGTCTAA AATCCCTTA  
 6961 ATCGGCCTCC TGTTTAGCTC CCGCTCTGAT TCCAACGAGG AAAGCACGTT ATACGTGTC  
 7021 GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCCGG GTGTGGTGGT  
 5 7081 TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT  
 7141 CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC  
 7201 TTTAGGGTTC CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA  
 7261 TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTT CGCCCTTGA CGTTGGAGTC  
 7321 CACGTTCTTT AATAGTGGAC TCTTGTCCA AACTGGAACA ACACCAACCT CTTATCTCGGG  
 10 7381 CTATTCTTTT GATTATAAG GGATTTGCC GATTTGGAA CCACCATCAA ACAGGATTTT  
 7441 CGCCTGCTGG GGAAACCCAG CGTGGACCGC TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG  
 7501 AAGGGCAATC AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCCACCCCT GGATCCAAGC  
 7561 TTGCAGGTGG CACTTTCCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA  
 7621 TACATTCAAA TATGTATCCG CTCATGAGAC AATAACCCCTG ATAAATGCTT CAATAATATT  
 15 7681 GAAAAAGGAA GAGTATGAGT ATTCAACATT TCCGTGTCGC CCTTATTCCC TTTTTTGC  
 7741 CATTTCGCCT TCCTGTTTT GCTCACCCAG AAACGCTGGT GAAAGTAAA GATGCTGAAG  
 7801 ATCAGTTGGG CGCACGAGTG GGTACATCG AACTGGATCT CAACAGCGGT AAGATCCTG  
 7861 AGAGTTTCG CCCCAGAGAA CGTTTCCAA TGATGAGCAC TTTAAAGTT CTGCTATGTC  
 7921 ATACACTATT ATCCCGTATT GACGCCGGC AAGAGCAACT CGGTGCCGG GCGCGGTATT  
 7981 CTCAGAATGA CTTGGTTGAG TACTCACCAG TCACAGAAAA GCATCTTACG GATGGCATGA  
 8041 CAGTAAGAGA ATTATGCACT GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC  
 8101 TTCTGACAAC GATCGGAGGA CCGAAGGAGC TAACCGCTT TTTGCACAAC ATGGGGGATC  
 8161 ATGTAACTCG CCTTGATCGT TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC  
 8221 GTGACACCAC GATGCCGTGA GCAATGCCAA CAACGTTGCG CAAACTATTAA ACTGGCGAAC  
 8281 TACTTACTCT AGCTTCCGG CAACAAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGAG  
 8341 GACCACTTCT GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG  
 8401 GTGAGCGTGG GTCTCGCGGT ATCATTGCACT CACTGGGCC AGATGGTAAG CCCTCCCGTA  
 8461 TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG  
 8521 CTGAGATAGG TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA  
 30 8581 TACTTTAGAT TGATTTAAA CTTCATTTT AATTTAAAG GATCTAGGTG AAGATCCTT  
 8641 TTGATAATCT CATGACAAA ATCCCTTAAC GTGAGTTTC GTTCCACTGT ACGTAAGACCC  
 8701 CCCAAGCTTG TCGACTGAAT GGCGAATGGC GCTTTGCCGTG GTTCCGGCA CCAGAAGCGG  
 8761 TGCCGGAAAG CTGGCTGGAG TGCGATCTTC CTGAGGCCGA TACTGTCGTC GTCCCTCAA  
 8821 ACTGGCAGAT GCACGGTTAC GATGCGCCCA TCTACACCAA CGTAACCTAT CCCATTACGG  
 8881 TCAATCCGCC GTTTGTTCCC ACGGAGAATC CGACGGGTTG TTACTCGCTC ACATTTAATG  
 8941 TTGATGAAAG CTGGCTACAG GAAGGCCAGA CGCGAATTAT TTTTGATGGC GTTCCTATTG  
 9001 GTTAAAAAAT GAGCTGATTT AACAAAAATT TAACGCGAAT TTTAACAAAA TATTAACGTT  
 9061 TACAATTAA ATATTTGCTT ATACAATCTT CCTGTTTTG GGGCTTTCT GATTATCAAC  
 9121 CGGGGTACAT ATGATTGACA TGCTAGTTT ACGATTACCG TTCATCGATT CTCTTGTTG

9181 CTCCAGACTC TCAGGCAATG ACCTGATAGC CTTTGTAGAT CTCTAAAAAA TAGCTACCCCT  
9241 CTCCGGCATG AATTATTCAG CTAGAACGGT TGAATATCAT ATTGATGGTG ATTTGACTGT  
9301 CTCCGGCCTT TCTCACCCCTT TTGAATCTTT ACCTACACAT TACTCAGGCA TTGCATTAA  
9361 AATATATGAG GGTTCTAAAAA ATTTTTATCC TTGCCTTGAA ATAAAGGCTT CTCCCGCAAA  
9421 AGTATTACAG GGTCTATAATG TTTTGTTAC AACCGATTTA GCTTTATGCT CTGAGGCTTT  
9481 ATTGCTTAAT TTTGCTAATT CTTTGCCTTG CCTGTATGAT TTATTGGATG TT

Table 200: Enzymes that either cut 15 or more human GLGs or have 5+-base recognition in FR3

Typical entry:

REname	Recognition	#sites
	GLGid#:base#	GLGid#:base#.....

5

BstEII	Ggttnacc	2
1:	3	48: 3

There are 2 hits at base# 3

10

MaeIII	gtnac	36				
1:	4	2: 4	3: 4	4: 4	5: 4	6: 4
7:	4	8: 4	9: 4	10: 4	11: 4	37: 4
37:	58	38: 4	38: 58	39: 4	39: 58	40: 4
40:	58	41: 4	41: 58	42: 4	42: 58	43: 4
43:	58	44: 4	44: 58	45: 4	45: 58	46: 4
46:	58	47: 4	47: 58	48: 4	49: 4	50: 58

There are 24 hits at base# 4

15

Tsp45I	gtsac	33				
1:	4	2: 4	3: 4	4: 4	5: 4	6: 4
7:	4	8: 4	9: 4	10: 4	11: 4	37: 4
37:	58	38: 4	38: 58	39: 58	40: 4	40: 58
41:	58	42: 58	43: 4	43: 58	44: 4	44: 58
45:	4	45: 58	46: 4	46: 58	47: 4	47: 58
48:	4	49: 4	50: 58			

There are 21 hits at base# 4

20

HphI	tcacc	45				
1:	5	2: 5	3: 5	4: 5	5: 5	6: 5
7:	5	8: 5	11: 5	12: 5	12: 11	13: 5
14:	5	15: 5	16: 5	17: 5	18: 5	19: 5
20:	5	21: 5	22: 5	23: 5	24: 5	25: 5
26:	5	27: 5	28: 5	29: 5	30: 5	31: 5
32:	5	33: 5	34: 5	35: 5	36: 5	37: 5
38:	5	40: 5	43: 5	44: 5	45: 5	46: 5
47:	5	48: 5	49: 5			

There are 44 hits at base# 5

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## NlaIII CATG

26

1:	9	1:	42	2:	42	3:	9	3:	42	4:	9	
4:	42	5:	9	5:	42	6:	42	6:	78	7:	9	
7:	42	8:	21	8:	42	9:	42	10:	42	11:	42	
5	12:	57	13:	48	13:	57	14:	57	31:	72	38:	9
	48:	78		49:	78							

There are 11 hits at base# 42

There are 1 hits at base# 48 Could cause raggedness.

## BsaJI Ccnngg

37

1:	14	2:	14	5:	14	6:	14	7:	14	8:	14	
8:	65	9:	14	10:	14	11:	14	12:	14	13:	14	
14:	14	15:	65	17:	14	17:	65	18:	65	19:	65	
20:	65	21:	65	22:	65	26:	65	29:	65	30:	65	
15	33:	65	34:	65	35:	65	37:	65	38:	65	39:	65
	40:	65	42:	65	43:	65	48:	65	49:	65	50:	65
	51:	14										

There are 23 hits at base# 65

There are 14 hits at base# 14

20

## AluI AGct

42

1:	47	2:	47	3:	47	4:	47	5:	47	6:	47	
7:	47	8:	47	9:	47	10:	47	11:	47	16:	63	
23:	63	24:	63	25:	63	31:	63	32:	63	36:	63	
25	37:	47	37:	52	38:	47	38:	52	39:	47	39:	52
	40:	47	40:	52	41:	47	41:	52	42:	47	42:	52
	43:	47	43:	52	44:	47	44:	52	45:	47	45:	52
	46:	47	46:	52	47:	47	47:	52	49:	15	50:	47

There are 23 hits at base# 47

30

There are 11 hits at base# 52 Only 5 bases from 47

## B1pI GCtnagc

21

1:	48	2:	48	3:	48	5:	48	6:	48	7:	48	
8:	48	9:	48	10:	48	11:	48	37:	48	38:	48	
35	39:	48	40:	48	41:	48	42:	48	43:	48	44:	48
	45:	48	46:	48	47:	48						

There are 21 hits at base# 48

MwoI GCNNNNNnngc 19  
1: 48 2: 28 19: 36 22: 36 23: 36 24: 36  
25: 36 26: 36 35: 36 37: 67 39: 67 40: 67  
41: 67 42: 67 43: 67 44: 67 45: 67 46: 67

5 47: 67

There are 10 hits at base# 67

There are 7 hits at base# 36

DdeI Ctnag 71  
10 1: 49 1: 58 2: 49 2: 58 3: 49 3: 58  
3: 65 4: 49 4: 58 5: 49 5: 58 5: 65  
6: 49 6: 58 6: 65 7: 49 7: 58 7: 65  
8: 49 8: 58 9: 49 9: 58 9: 65 10: 49  
10: 58 10: 65 11: 49 11: 58 11: 65 15: 58

15 16: 58 16: 65 17: 58 18: 58 20: 58 21: 58  
22: 58 23: 58 23: 65 24: 58 24: 65 25: 58  
25: 65 26: 58 27: 58 27: 65 28: 58 30: 58  
31: 58 31: 65 32: 58 32: 65 35: 58 36: 58  
36: 65 37: 49 38: 49 39: 26 39: 49 40: 49

20 41: 49 42: 26 42: 49 43: 49 44: 49 45: 49  
46: 49 47: 49 48: 12 49: 12 51: 65

There are 29 hits at base# 58

There are 22 hits at base# 49 Only nine base from 58

There are 16 hits at base# 65 Only seven bases from 58

25

BglII Agatct 11  
1: 61 2: 61 3: 61 4: 61 5: 61 6: 61  
7: 61 9: 61 10: 61 11: 61 51: 47

There are 10 hits at base# 61

30

BstYI Rgatcy 12  
1: 61 2: 61 3: 61 4: 61 5: 61 6: 61  
7: 61 8: 61 9: 61 10: 61 11: 61 51: 47

There are 11 hits at base# 61

35

Hpy188I TCNGa 17  
1: 64 2: 64 3: 64 4: 64 5: 64 6: 64  
7: 64 8: 64 9: 64 10: 64 11: 64 16: 57  
20: 57 27: 57 35: 57 48: 67 49: 67

5 There are 11 hits at base# 64  
There are 4 hits at base# 57  
There are 2 hits at base# 67 Could be ragged.

MslI CAYNNnnRTG 44  
10 1: 72 2: 72 3: 72 4: 72 5: 72 6: 72  
7: 72 8: 72 9: 72 10: 72 11: 72 15: 72  
17: 72 18: 72 19: 72 21: 72 23: 72 24: 72  
25: 72 26: 72 28: 72 29: 72 30: 72 31: 72  
32: 72 33: 72 34: 72 35: 72 36: 72 37: 72

15 38: 72 39: 72 40: 72 41: 72 42: 72 43: 72  
44: 72 45: 72 46: 72 47: 72 48: 72 49: 72  
50: 72 51: 72

There are 44 hits at base# 72

20 BsiEI CGRYcg 23  
1: 74 3: 74 4: 74 5: 74 7: 74 8: 74  
9: 74 10: 74 11: 74 17: 74 22: 74 30: 74  
33: 74 34: 74 37: 74 38: 74 39: 74 40: 74  
41: 74 42: 74 45: 74 46: 74 47: 74

25 There are 23 hits at base# 74

EaeI Yggccr 23  
1: 74 3: 74 4: 74 5: 74 7: 74 8: 74  
9: 74 10: 74 11: 74 17: 74 22: 74 30: 74  
30 33: 74 34: 74 37: 74 38: 74 39: 74 40: 74  
41: 74 42: 74 45: 74 46: 74 47: 74

There are 23 hits at base# 74

EagI Cggccg 23  
35 1: 74 3: 74 4: 74 5: 74 7: 74 8: 74  
9: 74 10: 74 11: 74 17: 74 22: 74 30: 74

**33: 74    34: 74    37: 74    38: 74    39: 74    40: 74**  
**41: 74    42: 74    45: 74    46: 74    47: 74**

There are 23 hits at base# 74

5	HaeIII	GGcc		27								
	1:	75	3:	75	4:	75	5:	75	7:	75	8:	75
	9:	75	10:	75	11:	75	16:	75	17:	75	20:	75
	22:	75	30:	75	33:	75	34:	75	37:	75	38:	75
	39:	75	40:	75	41:	75	42:	75	45:	75	46:	75

*10*      47: 75      48: 63      49: 63

There are 25 hits at base# 75

Bst4C1 ACNgt 65°C 63 Sites There is a third isoschismes

**1: 86      2: 86      3: 86      4: 86      5: 86      6: 86**

<b>15</b>	<b>7:</b> 34	<b>7:</b> 86	<b>8:</b> 86	<b>9:</b> 86	<b>10:</b> 86	<b>11:</b> 86
	<b>12:</b> 86	<b>13:</b> 86	<b>14:</b> 86	<b>15:</b> 36	<b>15:</b> 86	<b>16:</b> 53
	<b>16:</b> 86	<b>17:</b> 36	<b>17:</b> 86	<b>18:</b> 86	<b>19:</b> 86	<b>20:</b> 53
	<b>20:</b> 86	<b>21:</b> 36	<b>21:</b> 86	<b>22:</b> 0	<b>22:</b> 86	<b>23:</b> 86
	<b>24:</b> 86	<b>25:</b> 86	<b>26:</b> 86	<b>27:</b> 53	<b>27:</b> 86	<b>28:</b> 36
<b>20</b>	<b>28:</b> 86	<b>29:</b> 86	<b>30:</b> 86	<b>31:</b> 86	<b>32:</b> 86	<b>33:</b> 36
	<b>33:</b> 86	<b>34:</b> 86	<b>35:</b> 53	<b>35:</b> 86	<b>36:</b> 86	<b>37:</b> 86
	<b>38:</b> 86	<b>39:</b> 86	<b>40:</b> 86	<b>41:</b> 86	<b>42:</b> 86	<b>43:</b> 86
	<b>44:</b> 86	<b>45:</b> 86	<b>46:</b> 86	<b>47:</b> 86	<b>48:</b> 86	<b>49:</b> 86
	<b>50:</b> 86	<b>51:</b> 0	<b>51:</b> 86			

25 There are 51 hits at base# 86 All the other sites are well away

HRYCHAILI AGNAT 63

1: 86 2: 86 3: 86 4: 86 5: 86 6: 86

7: 34      7: 86      8: 86      9: 86      10: 86      11: 86

30	12: 86	13: 86	14: 86	15: 36	15: 86	16: 53
	16: 86	17: 36	17: 86	18: 86	19: 86	20: 53
	20: 86	21: 36	21: 86	22: 0	22: 86	23: 86
	24: 86	25: 86	26: 86	27: 53	27: 86	28: 36
	28: 86	29: 86	30: 86	31: 86	32: 86	33: 36
35	33: 86	34: 86	35: 53	35: 86	36: 86	37: 86
	38: 86	39: 86	40: 86	41: 86	42: 86	43: 86

44: 86 45: 86 46: 86 47: 86 48: 86 49: 86

50: 86 51: 0 51: 86

There are 51 hits at base# 86

5 HinFI Gantc 43  
2: 2 3: 2 4: 2 5: 2 6: 2 7: 2  
8: 2 9: 2 9: 22 10: 2 11: 2 15: 2  
16: 2 17: 2 18: 2 19: 2 19: 22 20: 2  
21: 2 23: 2 24: 2 25: 2 26: 2 27: 2  
10 28: 2 29: 2 30: 2 31: 2 32: 2 33: 2  
33: 22 34: 22 35: 2 36: 2 37: 2 38: 2  
40: 2 43: 2 44: 2 45: 2 46: 2 47: 2  
50: 60

There are 38 hits at base# 2

15 MlyI GAGTCNNNNn 18  
2: 2 3: 2 4: 2 5: 2 6: 2 7: 2  
8: 2 9: 2 10: 2 11: 2 37: 2 38: 2  
40: 2 43: 2 44: 2 45: 2 46: 2 47: 2

20 There are 18 hits at base# 2

PleI gagtc 18  
2: 2 3: 2 4: 2 5: 2 6: 2 7: 2  
8: 2 9: 2 10: 2 11: 2 37: 2 38: 2  
25 40: 2 43: 2 44: 2 45: 2 46: 2 47: 2

There are 18 hits at base# 2

AciI Cccg 24  
2: 26 9: 14 10: 14 11: 14 27: 74 37: 62  
37: 65 38: 62 39: 65 40: 62 40: 65 41: 65  
30 42: 65 43: 62 43: 65 44: 62 44: 65 45: 62  
46: 62 47: 62 47: 65 48: 35 48: 74 49: 74

There are 8 hits at base# 62

There are 8 hits at base# 65

There are 3 hits at base# 14

35 There are 3 hits at base# 74

There are 1 hits at base# 26

There are 1 hits at base# 35

-"- Gcgg 11  
8: 91 9: 16 10: 16 11: 16 37: 67 39: 67  
40: 67 42: 67 43: 67 45: 67 46: 67

There are 7 hits at base# 67

5 There are 3 hits at base# 16

There are 1 hits at base# 91

BsiHKAI GWGCWc 20  
2: 30 4: 30 6: 30 7: 30 9: 30 10: 30

10 12: 89 13: 89 14: 89 37: 51 38: 51 39: 51  
40: 51 41: 51 42: 51 43: 51 44: 51 45: 51  
46: 51 47: 51

There are 11 hits at base# 51

15 Bsp1286I GDGCHc 20  
2: 30 4: 30 6: 30 7: 30 9: 30 10: 30  
12: 89 13: 89 14: 89 37: 51 38: 51 39: 51  
40: 51 41: 51 42: 51 43: 51 44: 51 45: 51  
46: 51 47: 51

20 There are 11 hits at base# 51

HgiAI GWGCWc 20  
2: 30 4: 30 6: 30 7: 30 9: 30 10: 30  
12: 89 13: 89 14: 89 37: 51 38: 51 39: 51  
25 40: 51 41: 51 42: 51 43: 51 44: 51 45: 51  
46: 51 47: 51

There are 11 hits at base# 51

BsoFI GCngc 26  
30 2: 53 3: 53 5: 53 6: 53 7: 53 8: 53  
8: 91 9: 53 10: 53 11: 53 31: 53 36: 36  
37: 64 39: 64 40: 64 41: 64 42: 64 43: 64  
44: 64 45: 64 46: 64 47: 64 48: 53 49: 53  
50: 45 51: 53

35 There are 13 hits at base# 53

There are 10 hits at base# 64

TseI Gcwgc 17  
2: 53 3: 53 5: 53 6: 53 7: 53 8: 53

9: 53 10: 53 11: 53 31: 53 36: 36 45: 64  
46: 64 48: 53 49: 53 50: 45 51: 53

There are 13 hits at base# 53

5 MnII gagg 34  
3: 67 3: 95 4: 51 5: 16 5: 67 6: 67  
7: 67 8: 67 9: 67 10: 67 11: 67 15: 67  
16: 67 17: 67 19: 67 20: 67 21: 67 22: 67  
23: 67 24: 67 25: 67 26: 67 27: 67 28: 67  
10 29: 67 30: 67 31: 67 32: 67 33: 67 34: 67  
35: 67 36: 67 50: 67 51: 67

There are 31 hits at base# 67

HpyCH4V TGca 34  
15 5: 90 6: 90 11: 90 12: 90 13: 90 14: 90  
15: 44 16: 44 16: 90 17: 44 18: 90 19: 44  
20: 44 21: 44 22: 44 23: 44 24: 44 25: 44  
26: 44 27: 44 27: 90 28: 44 29: 44 33: 44  
34: 44 35: 44 35: 90 36: 38 48: 44 49: 44  
20 50: 44 50: 90 51: 44 51: 52

There are 21 hits at base# 44

There are 1 hits at base# 52

AccI GTmkac 13 5-base recognition  
25 7: 37 11: 24 37: 16 38: 16 39: 16 40: 16  
41: 16 42: 16 43: 16 44: 16 45: 16 46: 16  
47: 16

There are 11 hits at base# 16

30 SacII CCGCgg 8 6-base recognition  
9: 14 10: 14 11: 14 37: 65 39: 65 40: 65  
42: 65 43: 65

There are 5 hits at base# 65

There are 3 hits at base# 14

35 TfiI Gawtc 24  
9: 22 15: 2 16: 2 17: 2 18: 2 19: 2  
19: 22 20: 2 21: 2 23: 2 24: 2 25: 2

26: 2 27: 2 28: 2 29: 2 30: 2 31: 2  
32: 2 33: 2 33: 22 34: 22 35: 2 36: 2

There are 20 hits at base# 2

5 BsmAI Nnnnnngagac 19  
15: 11 16: 11 20: 11 21: 11 22: 11 23: 11  
24: 11 25: 11 26: 11 27: 11 28: 11 28: 56  
30: 11 31: 11 32: 11 35: 11 36: 11 44: 87  
48: 87

10 There are 16 hits at base# 11

BpmI ctccag 19  
15: 12 16: 12 17: 12 18: 12 20: 12 21: 12  
22: 12 23: 12 24: 12 25: 12 26: 12 27: 12  
15 28: 12 30: 12 31: 12 32: 12 34: 12 35: 12  
36: 12

There are 19 hits at base# 12

XmnI GAANNnnttc 12  
20 37: 30 38: 30 39: 30 40: 30 41: 30 42: 30  
43: 30 44: 30 45: 30 46: 30 47: 30 50: 30

There are 12 hits at base# 30

BsrI NCcagt 12  
25 37: 32 38: 32 39: 32 40: 32 41: 32 42: 32  
43: 32 44: 32 45: 32 46: 32 47: 32 50: 32

There are 12 hits at base# 32

BanII GRGCYc 11  
30 37: 51 38: 51 39: 51 40: 51 41: 51 42: 51  
43: 51 44: 51 45: 51 46: 51 47: 51

There are 11 hits at base# 51

Ecl136I GAGctc 11  
35 37: 51 38: 51 39: 51 40: 51 41: 51 42: 51  
43: 51 44: 51 45: 51 46: 51 47: 51

There are 11 hits at base# 51

SacI GAGCTc 11

37: 51    38: 51    39: 51    40: 51    41: 51    42: 51

43: 51    44: 51    45: 51    46: 51    47: 51

There are 11 hits at base# 51

---

Table 206: Synthetic 3-23 FR3 of human heavy chains showing positions of possible cleavage sites

Sites engineered into the synthetic gene are shown in upper case DNA with the RE name between vertical bars (as in | XbaI |). RERSSs frequently found in GLGs are shown below the synthetic sequence with the name to the right (as in gtn ac=MaeIII(24), indicating that 24 of the 51 GLGs contain the site).

```

10
| Allowed DNA
15
| R F synthetic 3-23)
| |cgc|ttc| 6
| |cgn|tty|
| |agr|
| ga ntc = HinfI(38)
| ga gtc = PstI(18)
| ga wtc = Tfil(20)
| gtn ac = MaeIII(24)
| gts ac = Tsp45I(21)
| tc acc = HphI(44)

20
| -----
| -----FR3-----
| 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
| T I S R D N S K N T L Y L Q M
| |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg| 51
| allowed|acn|ath|tcn|cgn|gay|aay|tcn|aar|aay|acn|ttr|tay|ttr|car|atg|
| |agy|agr|  |agy|  |ctn|  |ctn|
| | gal|gac = BsmAI(16)  ag ct = AluI(23)
| c|tcc ag = BpmI(19)  g ctn agc = BlpI(21)
| |           g aan nnn ttc = XmnI(12)
| | XbaI |  tg ca = HpyCH4V(21)

30
| -----
| -----FR3----->|
| 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
| N S L R A E D T A V Y Y C A K
| |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa| 96
| allowed|aay|tcn|ttr|cgn|gcn|gar|gay|acn|gcn|gtn|tay|tay|tgy|gcn|aar|
| |agy|ctn|agr|  |  |
| | cc nng g = BsaJI(23)  ac ngt = Bst4CI(51)
| | aga tct = BglII(10)  |  ac ngt = HpyCH4III(51)
| | Rga tcy = BstYI(11)  |  ac ngt = Taai(51)
| |           c ayn nnn rtc = MsII(44)
| |           cg ryc g = BsiE(23)
| |           yg gcc r = EaeI(23)
| |           cg gcc g = EagI(23)
| |           lg gcc = HaeIII(25)
| |           gag g = MnII(31)|
| |           | PstI |
| AfII

```

Table 217: Human HC GLG FR1 Sequences

VH Exon - Nucleotide sequence alignment

VH1

5	1-02 CAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG GTC TCC TGC AAG GCT TCT GGA TAC ACC TTC ACC
	1-03 cag gtC cag ctT gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtT tcc tgc aag gct tct gga tac acc ttc act
	1-08 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtc tcc tgc aag gct tct gga tac acc ttc acc
10	1-18 cag gtT cag ctg gtg cag tct ggA gct gag gtg aag aag cct ggg gcc tca gtg aag gtc tcc tgc aag gct tct ggT tac acc ttT acc
	1-24 cag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtc tcc tgc aag gTt tcC gga tac acc Ctc act
	1-45 cag Atg cag ctg gtg cag tct ggg gct gag gtg aag aag Act ggg Tcc tca gtg aag gtT tcc tgc aag gct tcC gga tac acc ttc acc
15	1-46 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtT tcc tgc aag gcA tct gga tac acc ttc acc
	1-58 caA Atg cag ctg gtg cag tct ggg Cct gag gtg aag aag cct ggg Acc tca gtg aag gtc tcc tgc aag gct tct gga tTc acc ttT act
20	1-69 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag gtc tcc tgc aag gct tct gga GGc acc ttc aGc
	1-e cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag gtc tcc tgc aag gct tct gga GGc acc ttc aGc
	1-f Gag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gCT Aca gtg aaA Atc tcc tgc aag gTt tct gga tac acc ttc acc

VH2

30	2-05 CAG ATC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA CCC ACA CAG ACC CTC ACG CTG ACC TGC ACC TTC TCT GGG TTC TCA CTC AGC
	2-26 cag Gtc acc ttg aag gag tct ggt cct GTg ctg gtg aaa ccc aca Gag acc ctc acg ctg acc tgc acc Gtc tct ggg ttc tca ctc agc
	2-70 cag Gtc acc ttg aag gag tct ggt cct Gcg ctg gtg aaa ccc aca cag acc ctc acA ctg acc tgc acc ttc tct ggg ttc tca ctc agc

VH3

35	3-07 GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTC CAG CCT GGG GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGT
	3-09 gaA gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggC Agg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttt GAt
	3-11 Cag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc Aag cct ggA ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt
40	3-13 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga ctc tcc tgt gca gcc tct gga ttc acc ttC agt
	3-15 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA Aag cct ggg ggg tcc ctT aga ctc tcc tgt gca gcc tct gga ttc acT ttC agt
	3-20 gag gtg cag ctg gtg gag tct ggg gga ggT Gtg gtA cGg cct ggg ggg tcc ctg aga

Sequence Data from GenBank

5            ctc tcc tgt gca gcc tct gga ttc acc ttt GAt  
3-21        gag gtg cag ctg gtg gag tct ggg gga ggc Ctg gtc Aag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
3-23        gag gtg cag ctg Ttg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttt agC  
3-30        Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
3-30.3 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
10        3-30.5 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
3-33        Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga  
              ctc tcc tgt gca gcG tct gga ttc acc ttC agt  
3-43        gaA gtg cag ctg gtg gag tct ggg gga gTc Gtg gtA cag cct ggg ggg tcc ctg aga  
15        ctc tcc tgt gca gcc tct gga ttc acc ttt GAt  
3-48        gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
3-49        gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag ccA ggg Cgg tcc ctg aga  
              ctc tcc tgt Aca gcT tct gga ttc acc ttt Ggt  
20        3-53        gag gtg cag ctg gtg gag Act ggA gga ggc ttg Atc cag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct ggG ttc acc GtC agt  
3-64        gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
3-66        gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc GtC agt  
25        3-72        gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggA ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
3-73        gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aAa  
              ctc tcc tgt gca gcc tct ggG ttc acc ttC agt  
30        3-74        gag gtg cag ctg gtg gag tcC ggg gga ggc ttA gtT cag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc ttC agt  
3-d        gag gtg cag ctg gtg gag tct Cgg gga gTc ttG gtA cag cct ggg ggg tcc ctg aga  
              ctc tcc tgt gca gcc tct gga ttc acc GtC agt  
VH4  
35        4-04        CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT CCT TCG GGG ACC CTG TCC  
              CTC ACC TGC GCT GTC TCT GGT GGC TCC ATC AGC  
4-28        cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAC acc ctg tcc  
              ctc acc tgc gct gtc tct ggt TAc tcc atc agc  
4-30.1 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc  
40        ctc acc tgc Act gtc tct ggt ggc tcc atc agc  
4-30.2 cag Ctg cag ctg cag gag tcC ggc Tca gga ctg gtg aag cct tcA CAg acc ctg tcc  
              ctc acc tgc gct gtc tct ggt ggc tcc atc agc  
4-30.4 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc  
              ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-31 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc  
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-34 cag gtg cag ctA cag Cag tGg ggc Gca gga ctg Ttg aag cct tcg gAg acc ctg tcc  
ctc acc tgc gct gtc tAt ggt ggG tcc Ttc agT

5 4-39 cag Ctg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc  
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-59 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc  
ctc acc tgc Act gtc tct ggt ggc tcc atc agT

4-61 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc

10 4-b cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc  
ctc acc tgc gct gtc tct ggt Gtc agc

VH5

5-51 GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG AAG  
ATC TCC TGT AAG GGT TCT GGA TAC AGC TTT ACC

15 5-a gaA gtg cag ctg gtg cag tct gga gca gag gtg aaa aag ccc ggg gag tct ctg aGg  
atc tcc tgt aag ggt tct gga tac agc ttt acc

VH6

6-1 CAG GTA CAG CTG CAG CAG TCA GGT CCA GGA CTG GTG AAG CCC TCG CAG ACC CTC TCA  
CTC ACC TGT GCC ATC TCC GGG GAC AGT GTC TCT

20 VH7

7-4.1 CAG GTG CAG CTG GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC TCA GTG AAG  
GTT TCC TGC AAG GCT TCT GGA TAC ACC TTC ACT

Table 220: RERS sites in Human HC GLG FR1s where there are at least 20 GLGs cut

BsgI GTGCAG

71 (cuts 16/14 bases to right)

	1: 4	1: 13	2: 13	3: 4	3: 13	4: 13
	6: 13	7: 4	7: 13	8: 13	9: 4	9: 13
5	10: 4	10: 13	15: 4	15: 65	16: 4	16: 65
	17: 4	17: 65	18: 4	18: 65	19: 4	19: 65
	20: 4	20: 65	21: 4	21: 65	22: 4	22: 65
	23: 4	23: 65	24: 4	24: 65	25: 4	25: 65
	26: 4	26: 65	27: 4	27: 65	28: 4	28: 65
10	29: 4	30: 4	30: 65	31: 4	31: 65	32: 4
	32: 65	33: 4	33: 65	34: 4	34: 65	35: 4
	35: 65	36: 4	36: 65	37: 4	38: 4	39: 4
	41: 4	42: 4	43: 4	45: 4	46: 4	47: 4
	48: 4	48: 13	49: 4	49: 13	51: 4	

15 There are 39 hits at base# 4

There are 21 hits at base# 65

-"- ctgcac

9

	12: 63	13: 63	14: 63	39: 63	41: 63	42: 63
20	44: 63	45: 63	46: 63			

BbvI GCAGC

65

	1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
	10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
	17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
25	20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
	23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
	26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
	30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
	33: 6	33: 67	34: 6	34: 67	35: 6	35: 67
30	36: 6	36: 67	37: 6	38: 6	39: 6	40: 6
	41: 6	42: 6	43: 6	44: 6	45: 6	46: 6
	47: 6	48: 6	49: 6	50: 12	51: 6	

There are 43 hits at base# 6 Bolded sites very near sites  
listed below

35 There are 21 hits at base# 67

-"- gctgc

13

37: 9	<b>38:</b> 9	39: 9	40: 3	40: 9	41: 9
42: 9	<b>44:</b> 3	<b>44:</b> 9	<b>45:</b> 9	<b>46:</b> 9	<b>47:</b> 9

50: 9

There are 11 hits at base# 9

BsoFI GCngc

78

5	1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
	10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
	17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
	20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
	23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
10	26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
	30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
	33: 6	33: 67	34: 6	34: 67	35: 6	35: 67
	36: 6	36: 67	<u>37: 6</u>	<u>37: 9</u>	<u>38: 6</u>	<u>38: 9</u>
	39: 6	39: 9	<u>40: 3</u>	<u>40: 6</u>	<u>40: 9</u>	<u>41: 6</u>
15	41: 9	42: 6	42: 9	43: 6	<u>44: 3</u>	<u>44: 6</u>
	<u>44: 9</u>	<u>45: 6</u>	<u>45: 9</u>	<u>46: 6</u>	<u>46: 9</u>	<u>47: 6</u>
	<u>47: 9</u>	48: 6	49: 6	50: 9	50: 12	51: 6

There are 43 hits at base# 6 These often occur together.

There are 11 hits at base# 9

20 There are 2 hits at base# 3

There are 21 hits at base# 67

TseI Gcwgc

78

25	1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
	10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
	17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
	20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
	23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
	26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
30	30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
	33: 6	33: 67	34: 6	34: 67	35: 6	35: 67
	36: 6	36: 67	<u>37: 6</u>	<u>37: 9</u>	<u>38: 6</u>	<u>38: 9</u>
	<u>39: 6</u>	<u>39: 9</u>	<u>40: 3</u>	<u>40: 6</u>	<u>40: 9</u>	<u>41: 6</u>
	<u>41: 9</u>	<u>42: 6</u>	<u>42: 9</u>	43: 6	<u>44: 3</u>	<u>44: 6</u>
35	<u>44: 9</u>	<u>45: 6</u>	<u>45: 9</u>	<u>46: 6</u>	<u>46: 9</u>	<u>47: 6</u>
	<u>47: 9</u>	48: 6	49: 6	50: 9	50: 12	51: 6

There are 43 hits at base# 6 Often together.

There are 11 hits at base# 9

There are 2 hits at base# 3

There are 1 hits at base# 12

There are 21 hits at base# 67

5 MspAll CMGckg 48  
1: 7 3: 7 4: 7 5: 7 6: 7 7: 7  
8: 7 9: 7 10: 7 11: 7 15: 7 16: 7  
17: 7 18: 7 19: 7 20: 7 21: 7 22: 7  
23: 7 24: 7 25: 7 26: 7 27: 7 28: 7  
10 29: 7 30: 7 31: 7 32: 7 33: 7 34: 7  
35: 7 36: 7 37: 7 38: 7 39: 7 40: 1  
40: 7 41: 7 42: 7 44: 1 44: 7 45: 7  
46: 7 47: 7 48: 7 49: 7 50: 7 51: 7

There are 46 hits at base# 7

15 PvuII CAGctg 48  
1: 7 3: 7 4: 7 5: 7 6: 7 7: 7  
8: 7 9: 7 10: 7 11: 7 15: 7 16: 7  
17: 7 18: 7 19: 7 20: 7 21: 7 22: 7  
20 23: 7 24: 7 25: 7 26: 7 27: 7 28: 7  
29: 7 30: 7 31: 7 32: 7 33: 7 34: 7  
35: 7 36: 7 37: 7 38: 7 39: 7 40: 1  
40: 7 41: 7 42: 7 44: 1 44: 7 45: 7  
46: 7 47: 7 48: 7 49: 7 50: 7 51: 7

25 There are 46 hits at base# 7

There are 2 hits at base# 1

AluI AGct 54  
1: 8 2: 8 3: 8 4: 8 4: 24 5: 8  
30 6: 8 7: 8 8: 8 9: 8 10: 8 11: 8  
15: 8 16: 8 17: 8 18: 8 19: 8 20: 8  
21: 8 22: 8 23: 8 24: 8 25: 8 26: 8  
27: 8 28: 8 29: 8 29: 69 30: 8 31: 8  
32: 8 33: 8 34: 8 35: 8 36: 8 37: 8  
35 38: 8 39: 8 40: 2 40: 8 41: 8 42: 8  
43: 8 44: 2 44: 8 45: 8 46: 8 47: 8  
48: 8 48: 82 49: 8 49: 82 50: 8 51: 8

There are 48 hits at base# 8

There are 2 hits at base# 2

	DdeI	Ctnag		48		
	1: 26	1: 48	2: 26	2: 48	3: 26	3: 48
5	4: 26	4: 48	5: 26	5: 48	6: 26	6: 48
	7: 26	7: 48	8: 26	8: 48	9: 26	10: 26
	11: 26	12: 85	13: 85	14: 85	15: 52	16: 52
	17: 52	18: 52	19: 52	20: 52	21: 52	22: 52
	23: 52	24: 52	25: 52	26: 52	27: 52	28: 52
10	29: 52	30: 52	31: 52	32: 52	33: 52	35: 30
	35: 52	36: 52	40: 24	49: 52	51: 26	51: 48

There are 22 hits at base# 52 52 and 48 never together.

There are 9 hits at base# 48

There are 12 hits at base# 26 26 and 24 never together.

15

	HphI	tcacc		42		
	1: 86	3: 86	6: 86	7: 86	8: 80	11: 86
	12: 5	13: 5	14: 5	15: 80	16: 80	17: 80
	18: 80	20: 80	21: 80	22: 80	23: 80	24: 80
20	25: 80	26: 80	27: 80	28: 80	29: 80	30: 80
	31: 80	32: 80	33: 80	34: 80	35: 80	36: 80
	37: 59	38: 59	39: 59	40: 59	41: 59	42: 59
	43: 59	44: 59	45: 59	46: 59	47: 59	50: 59

There are 22 hits at base# 80 80 and 86 never together

25 There are 5 hits at base# 86

There are 12 hits at base# 59

	BssKI	Nccnngg		50		
	1: 39	2: 39	3: 39	4: 39	5: 39	7: 39
30	8: 39	9: 39	10: 39	11: 39	15: 39	16: 39
	17: 39	18: 39	19: 39	20: 39	21: 29	21: 39
	22: 39	23: 39	24: 39	25: 39	26: 39	27: 39
	28: 39	29: 39	30: 39	31: 39	32: 39	33: 39
	34: 39	35: 19	35: 39	36: 39	37: 24	38: 24
35	39: 24	41: 24	42: 24	44: 24	45: 24	46: 24
	47: 24	<u>48: 39</u>	<u>48: 40</u>	<u>49: 39</u>	<u>49: 40</u>	50: 24
	50: 73	51: 39				

There are 35 hits at base# 39 39 and 40 together twice.

There are 2 hits at base# 40

BsaJI Ccnngg						47
	1: 40	2: 40	3: 40	4: 40	5: 40	7: 40
	8: 40	9: 40	9: 47	10: 40	10: 47	11: 40
5	15: 40	18: 40	19: 40	20: 40	21: 40	22: 40
	23: 40	24: 40	25: 40	26: 40	27: 40	28: 40
	29: 40	30: 40	31: 40	32: 40	34: 40	35: 20
	35: 40	36: 40	37: 24	38: 24	39: 24	41: 24
	42: 24	44: 24	45: 24	46: 24	47: 24	<u>48: 40</u>
10	<u>48: 41</u>	<u>49: 40</u>	<u>49: 41</u>	50: 74	51: 40	

There are 32 hits at base# 40 40 and 41 together twice

There are 2 hits at base# 41

There are 9 hits at base# 24

There are 2 hits at base# 47

15

BstNI CCwgg						44
	PspGI ccwgg					
	ScrFI (\$M.HpaII) CCwgg					
	1: 40	2: 40	3: 40	4: 40	5: 40	7: 40
20	8: 40	9: 40	10: 40	11: 40	15: 40	16: 40
	17: 40	18: 40	19: 40	20: 40	21: 30	21: 40
	22: 40	23: 40	24: 40	25: 40	26: 40	27: 40
	28: 40	29: 40	30: 40	31: 40	32: 40	33: 40
	34: 40	35: 40	36: 40	37: 25	38: 25	39: 25
25	41: 25	42: 25	44: 25	45: 25	46: 25	47: 25
	50: 25	51: 40				

There are 33 hits at base# 40

ScrFI CCnngg						50
30	1: 40	2: 40	3: 40	4: 40	5: 40	7: 40
	8: 40	9: 40	10: 40	11: 40	15: 40	16: 40
	17: 40	18: 40	19: 40	20: 40	21: 30	21: 40
	22: 40	23: 40	24: 40	25: 40	26: 40	27: 40
	28: 40	29: 40	30: 40	31: 40	32: 40	33: 40
35	34: 40	35: 20	35: 40	36: 40	37: 25	38: 25
	39: 25	41: 25	42: 25	44: 25	45: 25	46: 25
	47: 25	48: 40	48: 41	49: 40	49: 41	50: 25
	50: 74	51: 40				

There are 35 hits at base# 40

There are 2 hits at base# 41

EcoO109I RGgnccy 34  
1: 43 2: 43 3: 43 4: 43 5: 43 6: 43  
5 7: 43 8: 43 9: 43 10: 43 15: 46 16: 46  
17: 46 18: 46 19: 46 20: 46 21: 46 22: 46  
23: 46 24: 46 25: 46 26: 46 27: 46 28: 46  
30: 46 31: 46 32: 46 33: 46 34: 46 35: 46  
36: 46 37: 46 43: 79 51: 43

10 There are 22 hits at base# 46 46 and 43 never together

There are 11 hits at base# 43

NlaIV GGNncc 71  
1: 43 2: 43 3: 43 4: 43 5: 43 6: 43  
7: 43 8: 43 9: 43 9: 79 10: 43 10: 79  
15 15: 46 15: 47 16: 47 17: 46 17: 47 18: 46  
18: 47 19: 46 19: 47 20: 46 20: 47 21: 46  
21: 47 22: 46 22: 47 23: 47 24: 47 25: 47  
26: 47 27: 46 27: 47 28: 46 28: 47 29: 47  
30: 46 30: 47 31: 46 31: 47 32: 46 32: 47  
20 33: 46 33: 47 34: 46 34: 47 35: 46 35: 47  
36: 46 36: 47 37: 21 37: 46 37: 47 37: 79  
38: 21 39: 21 39: 79 40: 79 41: 21 41: 79  
42: 21 42: 79 43: 79 44: 21 44: 79 45: 21  
45: 79 46: 21 46: 79 47: 21 51: 43

25 There are 23 hits at base# 47 46 & 47 often together

There are 17 hits at base# 46 There are 11 hits at base# 43

Sau96I Ggncc 70  
1: 44 2: 3 2: 44 3: 44 4: 44 5: 3 5: 44 6: 44  
7: 44 8: 22 8: 44 9: 44 10: 44 11: 3 12: 22 13: 22  
30 14: 22 15: 33 15: 47 16: 47 17: 47 18: 47 19: 47 20: 47  
21: 47 22: 47 23: 33 23: 47 24: 33 24: 47 25: 33 25: 47  
26: 33 26: 47 27: 47 28: 47 29: 47 30: 47 31: 33 31: 47  
32: 33 32: 47 33: 33 33: 47 34: 33 34: 47 35: 47 36: 47  
37: 21 37: 22 37: 47 38: 21 38: 22 39: 21 39: 22 41: 21  
35 41: 22 42: 21 42: 22 43: 80 44: 21 44: 22 45: 21 45: 22  
46: 21 46: 22 47: 21 47: 22 50: 22 51: 44

There are 23 hits at base# 47 These do not occur together.

There are 11 hits at base# 44

There are 14 hits at base# 22 These do occur together.  
There are 9 hits at base# 21

BsmAI GTCTCNnnnn 22  
5 1: 58 3: 58 4: 58 5: 58 8: 58 9: 58  
10: 58 13: 70 36: 18 37: 70 38: 70 39: 70  
40: 70 41: 70 42: 70 44: 70 45: 70 46: 70  
47: 70 48: 48 49: 48 50: 85

There are 11 hits at base# 70

10

-"- Nnnnnnngagac 27  
13: 40 15: 48 16: 48 17: 48 18: 48 20: 48  
21: 48 22: 48 23: 48 24: 48 25: 48 26: 48  
27: 48 28: 48 29: 48 30: 10 30: 48 31: 48  
15 32: 48 33: 48 35: 48 36: 48 43: 40 44: 40  
45: 40 46: 40 47: 40

There are 20 hits at base# 48

AvaII Ggwcc 44  
20 Sau96I (\$M.HaeIII) Ggwcc 44  
2: 3 5: 3 6: 44 8: 44 9: 44 10: 44  
11: 3 12: 22 13: 22 14: 22 15: 33 15: 47  
16: 47 17: 47 18: 47 19: 47 20: 47 21: 47  
22: 47 23: 33 23: 47 24: 33 24: 47 25: 33  
25 25: 47 26: 33 26: 47 27: 47 28: 47 29: 47  
30: 47 31: 33 31: 47 32: 33 32: 47 33: 33  
33: 47 34: 33 34: 47 35: 47 36: 47 37: 47  
43: 80 50: 22

There are 23 hits at base# 47 44 & 47 never together

30 There are 4 hits at base# 44

PpuMI RGgwccy 27  
6: 43 8: 43 9: 43 10: 43 15: 46 16: 46  
17: 46 18: 46 19: 46 20: 46 21: 46 22: 46  
35 23: 46 24: 46 25: 46 26: 46 27: 46 28: 46  
30: 46 31: 46 32: 46 33: 46 34: 46 35: 46  
36: 46 37: 46 43: 79

There are 22 hits at base# 46 43 and 46 never occur together.

There are 4 hits at base# 43

BsmFI GGGAC

3

8: 43 37: 46 50: 77

-"- gtccc 33

5 15: 48 16: 48 17: 48 1: 0 1: 0 20: 48  
21: 48 22: 48 23: 48 24: 48 25: 48 26: 48  
27: 48 28: 48 29: 48 30: 48 31: 48 32: 48  
33: 48 34: 48 35: 48 36: 48 37: 54 38: 54  
39: 54 40: 54 41: 54 42: 54 43: 54 44: 54  
10 45: 54 46: 54 47: 54

There are 20 hits at base# 48

There are 11 hits at base# 54

HinfI Gantc

80

15 8: 77 12: 16 13: 16 14: 16 15: 16 15: 56  
15: 77 16: 16 16: 56 16: 77 17: 16 17: 56  
17: 77 18: 16 18: 56 18: 77 19: 16 19: 56  
19: 77 20: 16 20: 56 20: 77 21: 16 21: 56  
21: 77 22: 16 22: 56 22: 77 23: 16 23: 56  
20 23: 77 24: 16 24: 56 24: 77 25: 16 25: 56  
25: 77 26: 16 26: 56 26: 77 27: 16 27: 26  
27: 56 27: 77 28: 16 28: 56 28: 77 29: 16  
29: 56 29: 77 30: 56 31: 16 31: 56 31: 77  
32: 16 32: 56 32: 77 33: 16 33: 56 33: 77  
25 34: 16 35: 16 35: 56 35: 77 36: 16 36: 26  
36: 56 36: 77 37: 16 38: 16 39: 16 40: 16  
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16  
48: 46 49: 46

There are 34 hits at base# 16

30

TfiI Gawtc

21

8: 77 15: 77 16: 77 17: 77 18: 77 19: 77  
20: 77 21: 77 22: 77 23: 77 24: 77 25: 77  
26: 77 27: 77 28: 77 29: 77 31: 77 32: 77  
35 33: 77 35: 77 36: 77

There are 21 hits at base# 77

MlyI GAGTC 38  
12: 16 13: 16 14: 16 15: 16 16: 16 17: 16  
18: 16 19: 16 20: 16 21: 16 22: 16 23: 16  
24: 16 25: 16 26: 16 27: 16 27: 26 28: 16  
5 29: 16 31: 16 32: 16 33: 16 34: 16 35: 16  
36: 16 36: 26 37: 16 38: 16 39: 16 40: 16  
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16  
48: 46 49: 46

There are 34 hits at base# 16

10

-"- GACTC 21  
15: 56 16: 56 17: 56 18: 56 19: 56 20: 56  
21: 56 22: 56 23: 56 24: 56 25: 56 26: 56  
27: 56 28: 56 29: 56 30: 56 31: 56 32: 56

15

There are 21 hits at base# 56

PleI gagtc 38  
12: 16 13: 16 14: 16 15: 16 16: 16 17: 16  
18: 16 19: 16 20: 16 21: 16 22: 16 23: 16  
24: 16 25: 16 26: 16 27: 16 27: 26 28: 16  
29: 16 31: 16 32: 16 33: 16 34: 16 35: 16  
36: 16 36: 26 37: 16 38: 16 39: 16 40: 16  
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16  
25 48: 46 49: 46

There are 34 hits at base# 16

-"- gactc 21  
15: 56 16: 56 17: 56 18: 56 19: 56 20: 56  
21: 56 22: 56 23: 56 24: 56 25: 56 26: 56  
30 27: 56 28: 56 29: 56 30: 56 31: 56 32: 56  
33: 56 35: 56 36: 56

There are 21 hits at base# 56

AlwNI CAGNNNctg 26  
15: 68 16: 68 17: 68 18: 68 19: 68 20: 68  
35 21: 68 22: 68 23: 68 24: 68 25: 68 26: 68  
27: 68 28: 68 29: 68 30: 68 31: 68 32: 68  
33: 68 34: 68 35: 68 36: 68 39: 46 40: 46  
41: 46 42: 46

There are 22 hits at base# 68

SCANNED, # S

Table 255: Analysis of frequency of matching RE adaptors in actual V genes

A: H<sub>y</sub>CH<sub>4</sub>V in HC at bases 35-56

Id	Probe	dotted probe
20	6-1 agttctccTGCAgtgactc	agttctccTGCAgtgactc
	3-11 cactgtatCTGCAaatgaacag	cac.g.at.....aa.....ag
	3-09 ccctgtatCTGCAaatgaacag	ccc.g.at.....aa.....ag
	5-51 ccgcctacCTGCAGtgaggcag	ccgc..a.....tg..g.ag
	3-15 cgctgtatCTGCAaatgaacag	c..c.g.at.....aa.....ag
	7-4..1 cggcatatCTGCAGatctgcag	c.gca.at.....a.ctg.ag
	3-73 cggcgtatCTGCAaatgaacag	c.gcg.at.....aa.....ag
	5-a ctgcctacCTGCAGtgaggcag	ctgc..a.....tg..g.ag
25	3-49 tcgcctatCTGCAaatgaacag	tcgc..at.....aa.....ag

Seqs with the expected RE site only.....1004

(Counts only cases with 4 or fewer mismatches)

Seqs with only an unexpected site.....0

Seqs with both expected and unexpected.... 48

(Counts only cases with 4 or fewer mismatches)

Seqs with no sites.....0

#### B: BlpI in HC

	Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	Name
<i>10</i>	1	<b>133</b>	<b>73</b>	<b>16</b>	<b>11</b>	<b>13</b>	<b>6</b>	<b>9</b>	<b>1</b>	<b>4</b>	<b>0</b>	<b>119</b>	<b>1-58</b>
	2	14	11	1	0	0	0	0	1	0	1	12	1-02
	3	34	17	8	2	6	1	0	0	0	0	0	1-18
	4	120	50	32	16	10	9	1	1	1	0	2	5-51
	5	55	13	11	10	17	3	1	0	0	0	0	3-15
<i>15</i>	6	340	186	88	41	15	6	3	0	1	0	0	3303
	7	82	25	16	25	12	1	3	0	0	0	0	3-20
	8	3	0	2	0	1	0	0	0	0	0	0	74.1
	9	23	18	2	2	1	0	0	0	0	0	0	3-66
	10	2	1	0	1	0	0	0	0	0	0	0	3-64
<i>20</i>	11	<b>486</b>	<b>249</b>	<b>78</b>	<b>81</b>	<b>38</b>	<b>21</b>	<b>10</b>	<b>4</b>	<b>4</b>	<b>1</b>	<b>467</b>	<b>4301</b>
	12	16	6	3	1	0	1	1	3	1	0	1	6-1
	13	28	15	8	2	2	1	0	0	0	0	0	2-70
	14	2	0	2	0	0	0	0	0	0	0	0	2-26
												601	

	Name	Full sequence	Dot mode
1-58		acatggagCTGAGCCaggctgtgag	acatggagCTGAGCCaggctgtgag
1-02		acatggag <del>ctgtgag</del> ccaggctgtgag	.....g.....g.....
1-18		acatggagctgtgagccaggctgtgag	.....g.....g.....
5	5-51	acctgcagtggagcaggccctgtgaa	...c...c...tg.....a
	3-15	atctgcaaatgaaacaggccctgtgaa	.tc..c.aa..a.....a
	3-30.3	atctgcaaatgaaacaggccctgtgag	.tc..c.aa..a.....a
	3-20	atctgcaaatgaaacaggctgtgag	.tc..c.aa..a...t.....
	7-4.1	atctgcagatctgcaggccctaa	.tc..c..a.ct.....a.a
10	3-66	atcttcaaattgaaacaggccctgtgag	.tc.tc.aa..a.....a
	3-64	atcttcaaattggcgaggccctgtgag	.tc.tc.aa..g.....a
	4-30.1	ccctgaagctgtgactctgtgtgac	c.c..a.....tctg...c
	6-1	ccctgcagctgaaactctgtgtgac	c.c..c.....a.tctg...c
	2-70	tccttacaatgaccacaaatgaa	t.c.tacaa...c..a.a..ga
15	2-26	tccttaccatgaccacaaatgaa	t.c.tacca...c..a.a..ga

Seqs with the expected RE site only..... 597 (counting sequences with 4 or fewer mismatches)

Seqs with only an unexpected site..... 2

Seqs with both expected and unexpected.... 2

Seqs with no sites..... 686

### C: HpyCH4III, Bst4CI, or TaaI in HC

In scoring whether the RE site of interest is present, only ONS that have 4 or fewer mismatches are counted.

25 Number of sequences..... 1617

SCANNED, # 8

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	acngt
1	244	78	92	43	18	10	1	2	0	0	241	102#1,1
2	457	69	150	115	66	34	11	8	3	1	434	103#2,3
3	173	52	45	36	22	14	3	0	0	1	169	108#3
5	4	16	0	3	2	2	1	6	0	1	8	124#5,1
5	4	0	0	1	0	1	1	0	1	0	2	145#6
6	15	1	0	1	0	6	4	1	1	1	8	158#8
7	23	4	8	5	2	2	1	1	0	0	21	205#12
8	9	1	1	1	0	3	2	1	0	0	6	226#13
10	9	7	1	3	1	1	0	0	1	0	0	270#14
10	23	7	3	5	5	2	1	0	0	0	22	309#16,
11	35	5	10	7	6	3	3	0	1	0	31	313#18,
12	18	2	3	2	2	6	1	0	2	0	15	315#19
13	3	1	2	0	0	0	0	0	0	0	3	320#20
15	117	29	23	28	22	8	4	2	1	0	110	323#22
15	75	21	25	13	9	1	4	2	0	0	69	330#23,
16	14	2	2	2	3	0	3	1	1	0	9	349#29
17	2	0	0	1	0	0	1	0	0	0	1	372#33
18	1	0	0	1	0	0	0	0	0	0	1	373#34
20	19	2	0	0	0	0	0	0	0	2	0	3d#36
20	34	4	9	9	4	5	3	0	0	0	31	428#38
21	17	5	4	2	2	3	1	0	0	0	16	4302#40
22	75	15	17	24	7	10	1	1	0	0	73	439#44
23	40	14	15	4	5	1	0	1	0	0	39	551#48
24	213	26	56	60	42	20	7	2	0	0	204	5a#49
25	24	213	26	56	60	42	20	7	2	0	0	AA

Table 255 D

Seqs with both expected and unexpected....	8
Seqs with no sites.....	0

Analysis repeated using only 8 best REadaptors

	Id	Ntot	0	1	2	3	4	5	6	7	8+	
5	1	301	78	101	54	32	16	9	10	1	0	281 102#1 ccgtgtattactgtgcgagaga
	2	493	69	155	125	73	37	14	11	3	6	459 103#2 ctgtgtattactgtgcgagaga
	3	189	52	45	38	23	18	5	4	1	3	176 108#3 ccgtgtattactgtgcgagagg
	4	127	29	23	28	24	10	6	5	2	0	114 323#22 ccgtatattactgtgcgaaaga
10	5	78	21	25	14	11	1	4	2	0	0	72 330#23 ctgtgtattactgtgcgaaaga
	6	79	15	17	25	8	11	1	2	0	0	76 439#44 ctgtgtattactgtgcgagaca
	7	43	14	15	5	5	3	0	1	0	0	42 551#48 ccatgtattactgtgcgagaca
	8	307	26	63	72	51	38	24	14	13	6	250 5a#49 ccatgtattactgtgcgaga
	1	102#1		ccgtgtattactgtgcgagaga		ccgtgtattactgtgcgagaga						
15	2	103#2		ctgtgtattactgtgcgagaga		.	t.....					
	3	108#3		ccgtgtattactgtgcgagagg								g
	4	323#22		ccgtatattactgtgcgaaaga								a.....a...
	5	330#23		ctgtgtattactgtgcgaaaga								a.....a...
	6	439#44		ctgtgtattactgtgcgagaca								c.
20	7	551#48		ccatgtattactgtgcgagaca								a.....c.
	8	5a#49		ccatgtattactgtgcgagaAA								AA

Seqs with the expected RE site only.....1463 / 1617

Seqs with only an unexpected site..... 0

25 Seqs with both expected and unexpected.... 7

Seqs with no sites..... 0

Table 300: Kappa FR1 GLGs

	1	2	3	4	5	6	7	8	9	10	11	12	
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
!	13	14	15	16	17	18	19	20	21	22	23		
5	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	O12
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	O2
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	O18
10	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	O8
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	A20
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
15	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	A30
	AAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCT	GCC	ATG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!	L14
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCA	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!	L1
20	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCA	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!	L15
	GCC	ATC	CAG	TTG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	L4
	GCC	ATC	CAG	TTG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	
25	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	L18
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCT	TCC	GTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!	L5
	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCT	TCT	GTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!	L19
30	GAC	ATC	CAG	TTG	ACC	CAG	TCT	CCA	TCC	TTC	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	L8
	GCC	ATC	CGG	ATG	ACC	CAG	TCT	CCA	TTC	TCC	CTG	TCT	
	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!	L23
	GCC	ATC	CGG	ATG	ACC	CAG	TCT	CCA	TCC	TCA	TTC	TCT	
35	GCA	TCT	ACA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!	L9
	GTC	ATC	TGG	ATG	ACC	CAG	TCT	CCA	TCC	TTA	CTC	TCT	

GCA TCT ACA GGA GAC AGA GTC ACC ATC AGT TGT ! L24  
GCC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT  
GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L11  
GAC ATC CAG ATG ACC CAG TCT CCT TCC ACC CTG TCT  
5 GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L12  
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC  
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O11  
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC  
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O1  
10 GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC  
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A17  
GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC  
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A1  
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC  
15 GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A18  
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC  
GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A2  
GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC  
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A19  
20 GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC  
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A3  
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC TCA CCT  
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A23  
GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT  
25 TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A27  
GAA ATT GTG TTG ACG CAG TCT CCA GCC ACC CTG TCT  
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A11  
GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT  
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L2  
30 GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT  
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L16  
GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT  
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L6  
GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT  
35 TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L20  
GAA ATT GTA ATG ACA CAG TCT CCA GCC ACC CTG TCT

TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L25  
GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT  
GTG TCT CTG GGC GAG AGG GCC ACC ATC AAC TGC ! B3  
GAA ACG ACA CTC ACG CAG TCT CCA GCA TTC ATG TCA  
5 GCG ACT CCA GGA GAC AAA GTC AAC ATC TCC TGC ! B2  
GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT  
GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC ! A26  
GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT  
GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC ! A10  
10 GAT GTT GTG ATG ACA CAG TCT CCA GCT TTC CTC TCT  
GTG ACT CCA GGG GAG AAA GTC ACC ATC ACC TGC ! A14

Table 302 RERS sites found in Human Kappa FR1 GLGS

		MsII	FokI	PflFI	BsrI	BsmAI	MnII	HpyCH4V
		-->	<--	-->				
VKI								
012	1-69	3	3	23	12	49	15	18
02	101-169	103	103	123	112	149	115	118
018	201-269	203	203	223	212	249	215	218
08	301-369	303	303	323	312	349	315	318
A20	401-469	403	403	423	412	449	415	418
A30	501-569	503	503	523	512	549	515	518
L14	601-669	603	603	612	649	615	618	647
L1	701-769	703	703	723	712	749	715	718
L15	801-869	803	803	823	812	849	815	818
L4	901-969	-	903	923	912	949	906	915
L18	1001-1069	-	1003	1012	1049	1006	1015	1018
L5	1101-1169	1103	-	1112	1149	1115	1118	1147
L19	1201-1269	1203	1203	1212	1249	1215	1218	1247
L8	1301-1369	-	1303	1323	1312	1349	1306	1315
L23	1401-1469	1403	1403	1408	1412	1449	1415	1418
L9	1501-1569	1503	1503	1508	1523	1512	1549	1515
L24	1601-1669	1603	1608	1623	1612	1649	1615	1618
L11	1701-1769	1703	1703	1723	1712	1749	1715	1718
L12	1801-1869	1803	1803	1812	1849	1815	1818	1847

10

15

20

36  
136  
236  
336  
436  
536  
636  
736  
836  
936  
1036  
1136  
1236  
1336  
1436  
1536  
1636  
1736  
1836

.....  
.....  
.....  
.....  
.....

		MsII	FoKI -->	<-->	PfIFI	BsRI	BsmAI	MnII	HpyCH 4V
<b>VKII</b>									
01	1901-1969	-	-	-	-	-	-	1956	-
01	2001-2069	-	-	-	-	-	-	2056	-
A17	2101-2169	-	-	2112	-	2118	-	2156	-
5	A1	2201-2269	-	2212	-	2218	-	2256	-
A18	2301-2369	-	-	-	-	-	-	2356	-
A2	2401-2469	-	-	-	-	-	-	2456	-
A19	2501-2569	-	-	2512	-	2518	-	2556	-
A3	2601-2669	-	-	2612	-	2618	-	2656	-
10	A23	2701-2769	-	-	-	-	-	2729	2756
<b>VKIII</b>									
A27	2801-2869	-	-	2812	-	2818	2839	2860	-
A11	2901-2969	-	-	2912	-	2918	2939	2960	-
12	3001-3069	-	-	3012	-	3018	3039	3060	-
15	L16	3101-3169	-	3112	-	3118	3139	3160	-
16	3201-3269	-	-	3212	-	3218	3239	3260	-

5

10

15

3301-3369 3401-3469 3501-3569 3601-3669 3701-3769 3801-3869 3901-3969

	MspI	FokI	<-->	PflFI	BsrI	BsmAI	MnlI	HpyCH4V
120	3301-3369	-	-	3312	-	3318 3339	3360	-
125	3401-3469	-	-	3412	-	3418 3439	3460	-
<i>VKIV</i>								
B3	3501-3569	3503	-	3512	3515	3518 3539	3551<	-
<i>VKV</i>								
B2	3601-3669	-	-	3649	-	3618	3647	-
<i>VKVI</i>								
A26	3701-3769	-	-	3712	-	3718	-	-
A10	3801-3869	-	-	3812	-	3818	-	-
<i>A14</i>								
10	3901-3969	-	-	3912	-	3918	3930>	-

Table 302 RERS sites found in Human Kappa FR1 GLGs, continued

	SfaNI	SfcI	HinfI	MlyI	MspI	HphI	HpaII	
	<-->			--->	Tsp45I	xx38 xx56 xx62	MspI	
				same sites		xx06 xx52		
<i>VKI</i>								
012	1-69	37	41	53	53	55	56	-
02	101-169	137	141	153	153	155	156	-
018	201-269	237	241	253	253	255	256	-

	Sf <sub>n</sub> I	SfcI	HinfI	M <sub>n</sub> I	<->	<->	Ma <sub>n</sub> II	HphI	HpaII
							Tsp45I	xx38	xx56
							same sites	xx62	MsP <sub>I</sub>
08	301-369	337	341	353,	353	355	355	356	xx52
A20	401-469	437	441	453	453	455	455	456	-
A30	501-569	537	541	553	553	555	555	556	-
L14	601-669	637	641	653	653	655	655	656	-
L1	701-769	737	741	753	753	755	755	756	-
L15	801-869	837	841	853	853	855	855	856	-
L4	901-969	937	941	953	953	955	955	956	-
L18	1001-1069	1037	1041	1053	1053	1055	1055	1056	-
L5	1101-1169	1137	1141	1153	1153	1155	1155	1156	-
L19	1201-1269	1237	1241	1253	1253	1255	1255	1256	-
L8	1301-1369	1337	1341	1353	1353	1355	1355	1356	-
L23	1401-1469	1437	1441	1453	1453	1455	1455	1456	1406
L9	1501-1569	1537	1541	1553	1553	1555	1555	1556	1506
L24	1601-1669	1637	1641	1653	1653	1655	1655	1656	-
L11	1701-1769	1737	1741	1753	1753	1755	1755	1756	-
L12	1801-1869	1837	1841	1853	1853	1855	1855	1856	-
VKit									
O11	1901-1969	-	-	1918	1918	1937	1938		1952
O1	2001-2069	-	-	2018	2018	2037	2038		2052
A17	2101-2169	-	-	2112	2112	2137	2138		2152
A1	2201-2269	-	-	2212	2212	2237	2238		2252

Enzyme restriction map of the *luciferase* gene

	Sf <sub>n</sub> NI	SfcI	HinfI	M <sub>n</sub> YI --->	<-- Tsp45I same sites	MaeII xx38 xx56 xx62	HpaII xx38 xx56 xx62	HpaII MspI xx06 xx52
A18	2301-2369	-	-	2318	2318	2337	2338	2352
A2	2401-2469	-	-	2418	2418	2437	2438	2452
A19	2501-2569	-	-	2512	2512	2537	2538	2552
A3	2601-2669	-	-	2612	2612	2637	2638	2652
5	A23	2701-2769	-	-	2718	2718	2737	2738*
	V <sub>KV1</sub>							
A27	2801-2869	-	-	-	-	-	-	-
A11	2901-2969	-	-	-	-	-	-	-
L2	3001-3069	-	-	-	-	-	-	-
10	L16	3101-3169	-	-	-	-	-	-
L6	3201-3269	-	-	-	-	-	-	-
L20	3301-3369	-	-	-	-	-	-	-
L25	3401-3469	-	-	-	-	-	-	-
	V <sub>KV2</sub>							
B3	3501-3569	-	-	3525	3525	-	-	-
	V <sub>KV3</sub>							
B2	3601-3669	-	-	3639	3639	-	-	-
	V <sub>KV4</sub>							
A26	3701-3769	-	-	3712	3739	3712	3737 3755	3756 3762
20	A10	3801-3869	-	-	3812	3839	3837 3855	3856 3862
	A14	3901-3969	-	-	3939	3939	3937 3955	3956 3962

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www.maths.uq.edu.au/~mccann/teach/ma3503/lectures/lec10.pdf

Table 302 RERS sites found in Human Kappa FR1, continued

	VKII	BsaII xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I	HaeII I	Tsp509I
					NaeI NgOMI V		
5	012	1-69	-	-	-	-	-
	02	101-169	-	-	-	-	-
	018	201-269	-	-	-	-	-
	08	301-369	-	-	-	-	-
	A20	401-469	-	-	-	-	-
	A30	501-569	-	-	-	-	-
10	L14	601-669	-	-	-	-	-
	L1	701-769	-	-	-	-	-
	L15	801-869	-	-	-	-	-
	L4	901-969	-	-	-	-	-
	L18	1001-1069	-	-	-	-	-
15	L5	1101-1169	-	-	-	-	-
	L19	1201-1269	-	-	-	-	-
	L8	1301-1369	-	-	-	-	-
	L23	1401-1469	-	-	-	-	-
	L9	1501-1569	-	-	-	-	-
	L24	1601-1669	-	-	-	-	-

	BsaJI xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44	BsrFI --> <--	HaeII Cac8I NaeI NgomI V	Tsp509I
L11	1701-1769	-	-	-	-	-
L12	1801-1869	-	-	-	-	-
<b>VIII</b>						
011	1901-1969	1942	1943	1944	1951	1954
5	01 2001-2069	2042	2043	2044	2051	2054
A17	2101-2169	2142	-	-	2151	2154
A1	2201-2269	2242	-	-	2251	2254
A18	2301-2369	2342	2343	-	2351	2354
A2	2401-2469	2442	2443	-	2451	2454
A19	2501-2569	2542	2543	2544	2551	2554
A3	2601-2669	2642	2643	2644	2651	2654
A23	2701-2769	2742	-	-	2751	2754
<b>IX</b>						
A27	2801-2869	2843	2822	2843	2820	2841
J5	A11 2901-2969	2943	2943	2920 2941	-	2803
L2	3001-3069	3043	3043	3041	-	2903
L16	3101-3169	3143	3143	3120 3141	-	-
L6	3201-3269	3243	3243	3220 3241	-	3203
L20	3301-3369	3343	3343	3320 3341	-	3303

酶切位点：BsaI, BssK1, BpmI, BsrFI, HaeII, Tsp509I  
 识别序列：xx29 xx42 xx43, xx22 xx30 xx43, xx20 xx41 xx44, xx20 xx41 xx44, xx20 xx41 xx44, xx20 xx41 xx44

	BsaI	BssK1 (NstNI)	BpmI	BsrFI	HaeII	Tsp509I
	xx29 xx42 xx43	xx22 xx30 xx43	xx20 xx41 xx44	--> --> <--	NaeI	
L25 3401-3469	3443	3443	3420 3441	-	-	3403
VRTV						
B3 3501-3569 3529		3530	3520	-	3554	
VRV						
B2 3601-3669			3643 3641	-		
VRVI						
A26 3701-3769	-		3720	-	-	3703
A10 3801-3869	-		3820	-	-	3803
A14 3901-3969	3943		3920 3941	-	-	-

Table 400 Lambda FR1 GLG sequences

! VL1

5            CAG TCT GTG CTG ACT CAG CCA CCC TCG GTG TCT GAA  
 GCC CCC AGG CAG AGG GTC ACC ATC TCC TGT ! 1a  
 cag tct gtg ctg acG cag ccG ccc tcA gtg tct gGG  
 gcc ccA Ggg cag agg gtc acc atc tcc tgC ! 1e  
 cag tct gtg ctg act cag cca ccc tcA gCg tct gGG  
 Acc ccc Ggg cag agg gtc acc atc tcT tgt ! 1c  
 cag tct gtg ctg act cag cca ccc tcA gCg tct gGG  
 10          Acc ccc Ggg cag agg gtc acc atc tcT tgt ! 1g  
 cag tct gtg Ttg acG cag ccG ccc tcA gtg tct gCG  
 gcc ccA GgA cag aAg gtc acc atc tcc tgC ! 1b

! VL2

15          CAG TCT GCC CTG ACT CAG CCT CCC TCC GCG TCC GGG  
 TCT CCT GGA CAG TCA GTC ACC ATC TCC TGC ! 2c  
 cag tct gcc ctg act cag cct cGc tcA gTg tcc ggg  
 tct cct gga cag tca gtc acc atc tcc tgc ! 2e  
 cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg  
 tct cct gga cag tcG Atc acc atc tcc tgc ! 2a2  
 cag tct gcc ctg act cag cct ccc tcc gTg tcc ggg  
 tct cct gga cag tca gtc acc atc tcc tgc ! 2d  
 cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg  
 tct cct gga cag tcG Atc acc atc tcc tgc ! 2b2

! VL3

25          TCC TAT GAG CTG ACT CAG CCA CCC TCA GTG TCC GTG  
 TCC CCA GGA CAG ACA GCC AGC ATC ACC TGC ! 3r  
 tcc tat gag ctg act cag cca cTc tca gtg tcA gtg  
 Gcc cTG gga cag acG gcc agG atT acc tgT ! 3j  
 tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg  
 tcc cca gga caA acG gcc agG atc acc tgc ! 3p  
 tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg  
 tcc cTa gga cag aTG gcc agG atc acc tgc ! 3a  
 tcT tCt gag ctg act cag GAC ccT GcT gtg tcT gtg  
 Gcc TTG gga cag aca gTc agG atc acA tgc ! 3l

5

tcc tat gTg ctg act cag cca ccc tca gtg tcA gtg  
GCC cca gga Aag acG gcc agG atT acc tgT ! 3h  
tcc tat gag ctg acA cag cTa ccc tcG gtg tcA gtg  
tcc cca gga cag aca gcc agG atc acc tgc ! 3e  
tcc tat gag ctg aTG cag cca ccc tcG gtg tcA gtg  
tcc cca gga cag acG gcc agG atc acc tgc ! 3m  
tcc tat gag ctg acA cag cca Tcc tca gtg tcA gtg  
tcT ccG gga cag aca gcc agG atc acc tgc ! v2-19

! VL4

10 CTG CCT GTG CTG ACT CAG CCC CCG TCT GCA TCT GCC  
TTG CTG GGA GCC TCG ATC AAG CTC ACC TGC ! 4c  
cAg cct gtg ctg act caA TcA TcC tct gcC tct gCT  
tCC ctg gga Tcc tcg Gtc aag ctc acc tgc ! 4a  
cAg cTt gtg ctg act caA TcG ccC tct gcC tct gcc  
tCC ctg gga gcc tcg Gtc aag ctc acc tgc ! 4b

15 ! VL5

CAG CCT GTG CTG ACT CAG CCA CCT TCC TCC TCC GCA  
TCT CCT GGA GAA TCC GCC AGA CTC ACC TGC ! 5e  
cag Gct gtg ctg act cag ccG Gct tcc CTc tcT gca  
tct cct gga gCa tcA gcc agT ctc acc tgc ! 5c  
cag cct gtg ctg act cag cca Tct tcc CAT tcT gca  
tct Tct gga gCa tcA gTc aga ctc acc tgc ! 5b

20 ! VL6

AAT TTT ATG CTG ACT CAG CCC CAC TCT GTG TCG GAG  
TCT CCG GGG AAG ACG GTA ACC ATC TCC TGC ! 6a

25 ! VL7

CAG ACT GTG GTG ACT CAG GAG CCC TCA CTG ACT GTG  
TCC CCA GGA GGG ACA GTC ACT CTC ACC TGT ! 7a  
cag Gct gtg gtg act cag gag ccc tca ctg act gtg  
tcc cca gga ggg aca gtc act ctc acc tgt ! 7b

30 ! VL8

CAG ACT GTG GTG ACC CAG GAG CCA TCG TTC TCA GTG  
TCC CCT GGA GGG ACA GTC ACA CTC ACT TGT ! 8a

! VL9

CAG CCT GTG CTG ACT CAG CCA CCT TCT GCA TCA GCC  
TCC CTG GGA GCC TCG GTC ACA CTC ACC TGC ! 9a

! VL10

5

CAG GCA GGG CTG ACT CAG CCA CCC TCG GTG TCC AAG  
GGC TTG AGA CAG ACC GCC ACA CTC ACC TGC ! 10a

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Table 405 RERSs found in human lambda FR1 GLGs

! There are 31 lambda GLGs

MlyI NnnnnnGACTC 25

1:	6	3:	6	4:	6	6:	6	7:	6	8:	6	
5	9:	6	10:	6	11:	6	12:	6	15:	6	16:	6
	20:	6	21:	6	22:	6	23:	6	23:	50	24:	6
	25:	6	25:	50	26:	6	27:	6	28:	6	30:	6
	31:	6										

There are 23 hits at base# 6

10

-"- GAGTCNNNNNn 1

26:	34
-----	----

MwoI GCNNNNNnngc 20

15	1:	9	2:	9	3:	9	4:	9	11:	9	11:	56
	12:	9	13:	9	14:	9	16:	9	17:	9	18:	9
	19:	9	20:	9	23:	9	24:	9	25:	9	26:	9
	30:	9	31:	9								

There are 19 hits at base# 9

20 Hinfl Gantc

27

1:	12	3:	12	4:	12	6:	12	7:	12	8:	12	
	9:	12	10:	12	11:	12	12:	12	15:	12	16:	12
	20:	12	21:	12	22:	12	23:	12	23:	46	23:	56
	24:	12	25:	12	25:	56	26:	12	26:	34	27:	12
25	28:	12	30:	12	31:	12						

There are 23 hits at base# 12

PleI gactc 25

1:	12	3:	12	4:	12	6:	12	7:	12	8:	12	
	9:	12	10:	12	11:	12	12:	12	15:	12	16:	12
30	20:	12	21:	12	22:	12	23:	12	23:	56	24:	12
	25:	12	25:	56	26:	12	27:	12	28:	12	30:	12
	31:	12										

There are 23 hits at base# 12

35 -"- gagtc

1

26:	34
-----	----

DdeI	Ctnag		32		
1: 14	2: 24	3: 14	3: 24	4: 14	4: 24
5: 24	6: 14	7: 14	7: 24	8: 14	9: 14
10: 14	11: 14	11: 24	12: 14	12: 24	15: 5
15: 14	16: 14	16: 24	19: 24	20: 14	23: 14
24: 14	25: 14	26: 14	27: 14	28: 14	29: 30
30: 14	31: 14				

There are 21 hits at base# 14

10

BsaJI	Ccnngg		38		
1:	23	1:	40	2:	39
4:	39	4:	40	5:	39
13:	23	13:	39	14:	23
17:	23	17:	39	18:	23
21:	47	22:	38	22:	39
28:	39	29:	14	29:	39
31:	23	31:	32	30:	38
				30:	39
				30:	47

There are 17 hits at base# 39

20 There are 5 hits at base# 38

There are 5 hits at base# 40 Makes cleavage ragged.

MnII	cctc		35		
1:	23	2:	23	3:	23
6:	23	7:	19	8:	23
11:	23	13:	23	14:	23
19:	23	20:	47	21:	23
22:	29	22:	35	22:	47
27:	23	28:	23	30:	35
				30:	47
				31:	23

There are 21 hits at base# 23

30 There are 3 hits at base# 19

There are 3 hits at base# 29

There are 1 hits at base# 26

There are 1 hits at base# 27 These could make cleavage ragged

-"- gagg

7

**35**      1: 48      2: 48      3: 48      4: 48      27: 44      28: 44

29: 44

BssKI Nccngg 39  
1: 40 2: 39 3: 39 3: 40 4: 39 4: 40  
5 5: 39 6: 31 6: 39 7: 31 7: 39 8: 39  
9: 31 9: 39 10: 39 11: 39 12: 38 12: 52  
13: 39 13: 52 14: 52 16: 39 16: 52 17: 39  
17: 52 18: 39 18: 52 19: 39 19: 52 21: 38  
22: 38 23: 39 24: 39 26: 39 27: 39 28: 39

10 29: 14 29: 39 30: 38

There are 21 hits at base# 39

There are 4 hits at base# 38

There are 3 hits at base# 31

There are 3 hits at base# 40 Ragged

15

BstNI CCwgg 30  
1: 41 2: 40 5: 40 6: 40 7: 40 8: 40  
9: 40 10: 40 11: 40 12: 39 12: 53 13: 40  
13: 53 14: 53 16: 40 16: 53 17: 40 17: 53  
20 18: 40 18: 53 19: 53 21: 39 22: 39 23: 40  
24: 40 27: 40 28: 40 29: 15 29: 40 30: 39

There are 17 hits at base# 40

There are 7 hits at base# 53

There are 4 hits at base# 39

25 There are 1 hits at base# 41 Ragged

PspGI ccwgg 30  
1: 41 2: 40 5: 40 6: 40 7: 40 8: 40  
9: 40 10: 40 11: 40 12: 39 12: 53 13: 40  
30 13: 53 14: 53 16: 40 16: 53 17: 40 17: 53  
18: 40 18: 53 19: 53 21: 39 22: 39 23: 40  
24: 40 27: 40 28: 40 29: 15 29: 40 30: 39

There are 17 hits at base# 40

There are 7 hits at base# 53

35 There are 4 hits at base# 39

There are 1 hits at base# 41

ScrFI CCngg

39

1: 41 2: 40 3: 40 3: 41 4: 40 4: 41  
5 5: 40 6: 32 6: 40 7: 32 7: 40 8: 40  
9: 32 9: 40 10: 40 11: 40 12: 39 12: 53  
13: 40 13: 53 14: 53 16: 40 16: 53 17: 40  
17: 53 18: 40 18: 53 19: 40 19: 53 21: 39  
22: 39 23: 40 24: 40 26: 40 27: 40 28: 40  
10 29: 15 29: 40 30: 39

There are 21 hits at base# 40

There are 4 hits at base# 39

There are 3 hits at base# 41

15 MaeIII gtnac

16

1: 52 2: 52 3: 52 4: 52 5: 52 6: 52  
7: 52 9: 52 26: 52 27: 10 27: 52 28: 10  
28: 52 29: 10 29: 52 30: 52

There are 13 hits at base# 52

20

Tsp45I gtsac

15

1: 52 2: 52 3: 52 4: 52 5: 52 6: 52  
7: 52 9: 52 27: 10 27: 52 28: 10 28: 52  
29: 10 29: 52 30: 52

25 There are 12 hits at base# 52

HphI tcacc

26

1: 53 2: 53 3: 53 4: 53 5: 53 6: 53  
7: 53 8: 53 9: 53 10: 53 11: 59 13: 59  
30 14: 59 17: 59 18: 59 19: 59 20: 59 21: 59  
22: 59 23: 59 24: 59 25: 59 27: 59 28: 59  
30: 59 31: 59

There are 16 hits at base# 59

There are 10 hits at base# 53

35

BspMI ACCTGCNNNNn                    14  
11: 61    13: 61    14: 61    17: 61    18: 61    19: 61  
20: 61    21: 61    22: 61    23: 61    24: 61    25: 61  
30: 61    31: 61

5 There are 14 hits at base# 61 Goes into CDR1

---

Table 500: h3401-h2 captured Via CJ with BsmAI

	!	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
	!	S	A	Q	D	I	Q	M	T	Q	S	P	A	T	L	S	
		a	GT	GCA	Caa	gac	atc	cag	atg	acc	cag	tct	cca	gcc	acc	ctg	tct
5	!	ApaLI... L25,L6,L20,L2,L16,A11 ! Extender.....Bridge...															
	!	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
10	!	V	S	P	G	E	R	A	T	L	S	C	R	A	S	Q	
		gtg	tct	cca	ggg	gaa	agg	gcc	acc	ctc	tcc	tgc	agg	gcc	agt	cag	
	!	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	
15	!	S	V	S	N	N	L	A	W	Y	Q	Q	K	P	G	Q	
		agt	gtt	agt	aac	aac	tta	gcc	tgg	tac	cag	cag	aaa	cct	ggc	cag	
	!	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
	!	V	P	R	L	L	I	Y	G	A	S	T	R	A	T	D	
		gtt	ccc	agg	ctc	ctc	atc	tat	ggt	gca	tcc	acc	agg	gcc	act	gat	
20	!	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	
	!	I	P	A	R	F	S	G	S	G	S	G	T	D	F	T	
		atc	cca	gcc	agg	ttc	agt	ggc	agt	ggg	tct	ggg	aca	gac	ttc	act	
25	!	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	
	!	L	T	I	S	R	L	E	P	E	D	F	A	V	Y	Y	
		ctc	acc	atc	agc	aga	ctg	gag	cct	gaa	gat	ttt	gca	gtg	tat	tac	
30	!	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	
	!	C	Q	R	Y	G	S	S	P	G	W	T	F	G	Q	G	
		tgt	cag	cgg	tat	ggt	agc	tca	ccg	ggg	tgg	acg	ttc	ggc	caa	ggg	
	!	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	
35	!	T	K	V	E	I	K	R	T	V	A	A	P	S	V	F	
		acc	aag	gtg	gaa	atc	aaa	cga	act	gtg	gct	gca	cca	tct	gtc	ttc	
	!	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	
	!	I	F	P	P	S	D	E	Q	L	K	S	G	T	A	S	
		atc	ttc	ccg	cca	tct	gat	gag	cag	ttg	aaa	tct	gga	act	gcc	tct	
40	!	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	
	!	V	V	C	L	L	N	N	F	Y	P	R	E	A	K	V	
		gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	tat	ccc	aga	gag	gcc	aaa	gta	

```

! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
! Q   W   K   V   D   N   A   L   Q   S   G   N   S   Q   E
cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag

5 ! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
! S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S
agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc agc

10 ! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
! S   T   L   T   L   S   K   A   D   Y   E   K   H   K   V
agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc

15 ! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
! Y   A   C   E   V   T   H   Q   G   L   S   S   P   V   T
tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg cct gtc aca

20 ! 211 212 213 214 215 216 217 218 219 220 221 222 223
! K   S   F   N   K   G   E   C   K   G   E   F   A
aag agc ttc aac aaa gga gag tgt aag ggc gaa ttc gc.....

```

Table 501: h3401-d8 KAPPA captured with CJ and *BsmAI*

```

25 ! 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
! S A Q D I Q M T Q S P A T L S
! aGT GCA Caa gac atc cag atg acc cag tct cct gcc acc ctg tct
! ApaLI...Extender.....@ gcc acc !
L25,L6,L20,L2,L16,A11

!
30 ! 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
! V S P G E R A T L S C R A S Q
! gtg tct cca ggt gaa aga gcc acc ctc tcc tgc agg gcc agt cag
! GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L2

35 ! 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
! N L L S N L A W Y Q Q K P G Q
! aat ctt ctc agc aac tta gcc tgg tac cag cag aaa cct ggc cag

40 ! 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
! A P R L L I Y G A S T G A I G
! gct ccc agg ctc ctc atc tat ggt gct tcc acc ggg gcc att ggt

45 ! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
! I P A R F S G S G S G T E F T
! atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gag ttc act

```

! 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90  
 ! L T I S S L Q S E D F A V Y F  
 ctc acc atc agc agc ctg cag tct gaa gat ttt gca gtg tat ttc  
  
 5 ! 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105  
 ! C Q Q Y G T S P P T F G G G T  
 tgt cag cag tat ggt acc tca ccg ccc act ttc ggc gga ggg acc  
  
 10 ! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120  
 ! K V E I K R T V A A P S V F I  
 aag gtg gag atc aaa cga act gtg gct gca cca tct gtc ttc atc  
  
 ! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135  
 ! F P P S D E Q L K S G T A S V  
 15 ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt  
  
 ! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150  
 ! V C P L N N F Y P R E A K V Q  
 gtg tgc ccg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag  
  
 20 ! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165  
 ! W K V D N A L Q S G N S Q E S  
 tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt  
  
 25 ! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180  
 ! V T E Q D N K D S T Y S L S S  
 gtc aca gag cag gac aac aag gac agc acc tac agc ctc agc agc  
  
 30 ! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195  
 ! T L T L S K V D Y E K H E V Y  
 acc ctg acg ctg agc aaa gta gac tac gag aaa cac gaa gtc tac  
  
 ! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210  
 ! A C E V T H Q G L S S P V T K  
 35 gcc tgc gaa gtc acc cat cag ggc ctt agc tcg ccc gtc acg aag  
  
 ! 211 212 213 214 215 216 217 218 219 220 221 222 223  
 ! S F N R G E C K K E F V  
 agc ttc aac agg gga gag tgt aag aaa gaa ttc gtt t

40

Table 508 Human heavy chains bases 88.1 to 94.2

Number of sequences . . . . . 840

Id	Ntot	Number of Mismatches.....						Name	Sequence.....	Probe	Dot form.....
		0	1	2	3	4	5				
1	364	152	97	76	26	7	4	2	VHS881-1.1	gcgtgttattactgtgcgag	gctgtgttattactgtgcgag
2	265	150	60	33	13	5	4	0	VHS881-1.2	gcgtgttattactgtgcgag	..c.....
3	96	14	34	16	10	5	7	9	VHS881-2.1	gcgttatattactgtgcgag	..c...a.....
4	20	0	3	4	9	2	2	0	VHS881-4.1	gcgtgttattactgtacgag	..c.....a....
5	95	25	36	18	11	2	2	0	VHS881-9.1	ccatgttattactgtqcqac	..ca.....
	840	341	230	147	69	21	19	11			
		341	571	718	787	808	827	838	840		

2

88 89 90 91 92 93 94 95 Codon number as in Table 195  
 Recognition..... Stem..... Loop..... Stem.....

(VHS881-1.1)	5'-gtctgtgtat tact-gtgcgag	CACATccgtg	TTrggT	CACggatcTtg-3
(VHS881-1.2)	5'-gccgtgtat tact-gtgcgag	CACATccgtg	TTrggT	CACggatcTtg-3
(VHS881-2.1)	5'-gccgttatat tact-gtgcgag	CACATccgtg	TTrggT	CACggatcTtg-3
(VHS881-4.1)	5'-gccgtgtat tact-gtacgag	CACATccgtg	TTrggT	CACggatcTtg-3
(VHS881-9.1)	5'-occattgtat tact-otcogaat	CACATccata	TTrggT	CACggatcTtg-3

Fig. 2. Schematic diagram of the substrate cleavage site.

site of substrate cleavage

(FOK)act 5'-GACATccccTTCTT GACCATccT-3'

(VHEx81) 5'-AATAGTAGAC TgcAgtTgc TCAggCCCTTA AgctgttCAT ctgcAAgTAG-  
                  AGATGTTCT TAGAgtTgTC TCTAGACTTA gTgAAGcg-3',  
note that VHEx81 is the reverse complement of the ON below  
**[RC]** 5'-cgCttcacTaag-  
Scab.....  
Synthetic 3-23 as in Table 206

XbaI | aac| agc| TTA| AGG| gct| gag| gac| act| GCA| Gtc| tac| ctat| t-3  
XbaI | aac| agc| TTA| AGG| gct| gag| gac| act| GCA| Gtc| tac| ctat| t-3

35 ! (VHBAFF81)

5'-ccGCTtacGttaaG-  
 | TGT|AGA|gac|aac|tct|aat|act|ctc|tac|ttg|cag|atg|~  
 | aac|tac|TTA|AGg|gct|act|GCA|Gtc|tac|tat|tgt|gct|gag-3'  
 CCGTGGG

again about 10' above the ground at which height it  
is broken into two distinct parts.

(VH881PCR) 5'-cgCTtcAaAg TCT AGA | gac | aac | tct | aag | aat | act | ctg | tac | ttg | cag | atg | -  
| aac | agc | TTA | AGg | gct | gag | gac | ACT | GCA | Gtc | tac | tat | tgt Acg ag-3'

5 Table 512: Kappa: bases 12-30

ID	Ntot	0	1	2	3	4	5	6	Name	Sequence.....	Dot Form.....
1	84	40	21	20	1	2	0	0	SK12012	gaccaggctccatcc	gacccaggctccatcc
2	32	19	3	6	2	1	0	1	SK12A17	gactcagttccacttcc	...t.....ct...
3	26	17	8	1	0	0	0	0	SK12A27	gacgcaggctccaggcacc	...g.....gg.a..
4	40	21	18	1	0	0	0	0	SK12A11	gacgcaggctccaggcacc	...g.....g.a..
182	97	50	28	3	3	0	0	1			
	97	147	175	178	181	181	182				

VIRE adaptors:

(SZKB1230-012) [RC] Stem..... Loop. Stem..... Recognition.....  
 5'-CACATccTGT TTGTT CACGGATG TG ggggATggAGActggGTc-3'  
 5'-gaccaggatccatccc cacATcGG ACAA cACGGATG-3'

FOKL.

! (SzKB1230-A17)	[RC]	5'-ch <del>a</del> <u>ATccTg</u> T <del>T</del> TT 5'-gactc <u>agtctccacttcc</u> <u>cacatccgtg</u> AACAA <u>cacaggatgtg</u> -3' Recognition..... Stem..... Loop.	Stem..... Loop. Stem..... Recognition..... Stem..... Loop.
!	EokI	EokI	EokI

(SzKB1230-A27)	[RC]	5'-cacATccTGT TTGT CACGGATGTT ggTGCCTggAGActGcTC-3' 5'-gacgcagtctccaggacc <u>cACATGTT</u> AACAA <u>cACGGATGTT</u> -3'	Recognition.....	Stem.....	Loop.....
					Pri-T
					Ext-T

(SzKB1230-A11)	5'-cacATccTg TTgtT	cacggATgtg ggTggCTggACTgcTgc-3'
[RC]	5'-gacgagtctccaggccacc <u>cacatccTg</u> ACAA <u>acggATgtg</u> -3'	
	Recognition..... Stem.....	Recognition..... Loop..... Stem.....

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What happens in the upper strand:

	(SzKB1230-O12*)	5'-gac cca gtc tcc a-tc ctc c-3' [SzKB1230-A17*] 5' Site of cleavage in substrate
5	(SzKB1230-A17*)	5'-gac tca gtc tcc a-tc ctc c-3'
	(SzKB1230-A27*)	5'-gac gca gtc tcc a-gg cac c-3'
	(SzKB1230-A11*)	5'-gac gca gtc tcc a-gc cac c-3'
10	(kapextURE)	5'-ccTctactctTgTcAcAg <u>TgcACAA</u> gAc ATc cAG-3'   sense strand Scab.....ApalI.
15	(kapextUREPCR)	5'-ccTctactctTgTcAcAg <u>Tg</u> -3' Scab.....
20	(kabRO1UR) [RC]	5'-ggaggATggA ctggATgtCT TgtgcActgtT gacaAGAGTA gagg-3' (kabRO2UR) [RC]
	(kabRO2UR) [RC]	5'-cCTctactctTgTcAcAg <u>TgcACAA</u> gAc ATc CAG tcc a-tc ctc c-3' ON
	(kabRO3UR) [RC]	5'-ggAGAGTggA ctggATgtCT TgtgcActgtT gacaAGAGTA gagg-3'
	(kabRO3UR) [RC]	5'-cCTctactctTgTcAcAg <u>TgcACAA</u> gAc ATc CAG tcc a-tc ctc c-3' ON
	(kabRO4UR) [RC]	5'-ggTggCTggA ctggATgtCT TgtgcActgtT gacaAGAGTA gagg-3'
25	(kabRO4UR) [RC]	5'-cCTctactctTgTcAcAg <u>TgcACAA</u> gAc ATc CAG tcc a-gc cac c-3' ON

1978, P. H. Goss, with support from the U.S. Fish and Wildlife Service, prepared a report on the status of the species.

(VH881PCR) 5'-cqGCTtcfaag[TCT]AGAaac-3',  
 |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-  
 |aac|agg|TTA|AGg|gct|gag|gac|act|GCA|Gtc|tac|tat|tgt Acg ag-3'

Table 512: Kappa, bases 12-30

ID	Ntot	0	1	2	3	4	5	6	Name	Sequence.....	Dot Form.....
1	84	40	21	20	1	2	0	0	SK12012	gaccaggctccatccctcc	gaccaggctccatccctcc
2	32	19	3	6	2	1	0	1	SK12A17	gactcagtctccactctcc	...t.....ct....
3	26	17	8	1	0	0	0	0	SK12A27	gacgcaggctccaggacc	...g.....gg.a..
4	40	21	18	1	0	0	0	0	SK12A11	gacgcaggctccaggccacc	...g.....g.a..
	182	97	50	28	3	3	0	1			
	97	147	175	178	181	181	182				

## URE adapters:

! Stem..... Loop. Stem..... Recognition.....  
 ! (SZKB1230-012) 5'-CACATccgtg TTGTT cacggatgtg ggaggatggagActgggtc-3'  
 ! [RC] 5'-gacccagtctccatcctcc cacatcg ACAA cacggatgtg-3'

Recognition..... Stem..... loop. Stem.... Foki. Foki.

<p>(SzKB1230-A17)</p>	<p>[RC]</p>	<p>5'-cA<b>CatcgTg</b> TggTT CACGGATgTg ggAGA<b>Gtgc</b>AgtGAGTC-3' 5'-gactcagtc<b>ttccactctcc</b> <b>cacAtcgTg</b> AACAA <b>cACggATgtg-3'</b></p>	<p>Recognition..... Stem..... Loop. Stem..... Recognition..... Recognition..... Stem..... Stem..... Stem..... loop. Stem..... FokI..... FokI.</p>
-----------------------	-------------	--	---

(S2KB1230-A27)	[RC]	5'-cAcATccTg TtGTT 5'-gacggagtctccaggacc	cacggATGtg ggTgcctggagactcggtc-3' <u>cACAT<u>GGT</u></u> AACAA <u>cAC<u>GGATG</u>TG</u> -3'	Recognition..... Stem..... Loop..... Stem..... Recognition..... Stem.....
----------------	------	---	--	---

5 [SZKB1230-A11)	<p>Stem..... Loop. Stem..... Recognition.....</p> <p>5'-cA<b>C</b>A<u>T</u>ccTg TTTT acggATG Tg ggTggctggAgActgcTc-3'  <span style="border: 1px solid black; padding: 2px;">[RC]</span> 5'-gacgca<u>G</u>tc<u>C</u>aggccacc <b>cAC</b><u>T</u>ccTg AACAA <b>cAC</b><u>T</u>ccTg-3'</p> <p>Recognition..... Stem..... Stem..... loop. Stem.</p>
---------------------	--

new growth stage 4, early in the growth of  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_3$ ,  $\text{R}_4$ ,  $\text{R}_5$ ,  $\text{R}_6$ ,  $\text{R}_7$ ,  $\text{R}_8$ ,  $\text{R}_9$ ,  $\text{R}_{10}$ ,  $\text{R}_{11}$ ,  $\text{R}_{12}$ ,  $\text{R}_{13}$

What happens in the upper strand:

- 5' (SzKB1230-012\*) 5'-gac cca gtc|tcc a-tc ctc c-3'  
5 | Site of cleavage in substrate
- 5' (SzKB1230-A17\*) 5'-gac tca gtc|tcc a-tc ctc c-3'
- 10 (SzKB1230-A27\*) 5'-gac gca gtc|tcc a-gg cac c-3'
- (SzKB1230-A11\*) 5'-gac gca gtc|tcc a-gc cac c-3'
- (kapextURE) 5'-ccTctactctTgTcAcAGTccACAA gac ATC CAG-3' !sense strand  
Scab.....ApalI.
- 15 (kapextUREPCR) 5'-ccTctactctTgTcAcAGTg-3'  
Scab.....
- (kaBRO1UR) 5'-ggaggatgg ctggactgt Tgtgactgt gacaAGAGTA gAgg-3'  
[RC] 5'-ccTctactctTgTcAcAGTccAA GAC ATC CAG tcc a-tc ctc c-3' ON above is R.C. of this one
- 20 (kaBRO2UR) 5'-ggaggatgg ctggatgt Tgtgactgt Tgtgactgt gacaAGAGTA gAgg-3'  
[RC] 5'-ccTctactctTgTcAcAGTccAA GAC ATC CAG tcc a-tc ctc c-3' ON above is R.C. of this one
- (kaBRO3UR) 5'-ggggccgg ctggatgt Tgtgactgt gacaAGAGTA gAgg-3'  
[RC] 5'-ccTctactctTgTcAcAGTccAA GAC ATC CAG tcc a-gg cac c-3' ON above is R.C. of this one
- (kaBRO4UR) 5'-ggggccgg ctggatgt Tgtgactgt gacaAGAGTA gAgg-3'  
25 [RC] 5'-ccTctactctTgTcAcAGTccAA GAC ATC CAG tcc a-gc cac c-3' ON above is R.C. of this one  
Scab.....ApalI.

Table 515 Lambda URE adapters bases 13.3 to 19.3

What happens in the top strand:

! site of cleavage in the upper strand  
5 (VL133-2a2\*) 5'-g tct cct g|ga cag tcg atc  
! (VL133-31\*) 5'-g gcc ttg g|ga cag aca gtc  
! (VL133-2c\*) 5'-g tct cct g|ga cag tca gtc  
! 10 (VL133-1c\*) 5'-g gcc cca g|gg cag agg gtc  
!  
! The following Extenders and Bridges all encode the AA sequence of 2a2 for codons 1-15  
! 15 (ON\_LamEx133) 5'-ccTcTgAcTgAgT **gcA** cAg -  
!  
! 2 3 4 5 6 7 8 9 10 11 12  
AGt gCT TtA acC caA ccG gcT AGT gtT AGC ggT-  
!  
20 ! 13 14 15  
tCC ccG g ! 2a2  
!  
! 1 (ON\_LamB1-133) [RC] 5'-ccTcTgAcTgAgT **gcA** cAg -  
!  
25 ! 2 3 4 5 6 7 8 9 10 11 12  
AGt gCT TtA acC caA ccG gcT AGT gtT AGC ggT-  
!  
! 13 14 15  
tCC ccG g ga cag tcg at-3' ! 2a2 **N.B.** the actual seq is the  
30 ! reverse complement of the one shown.  
!  
(ON\_LamB2-133) [RC] 5'-ccTcTgAcTgAgT **gcA** cAg -  
!  
35 ! 2 3 4 5 6 7 8 9 10 11 12  
AGt gCT TtA acC caA ccG gcT AGT gtT AGC ggT-  
!  
! 13 14 15  
tCC ccG g ga cag aca gt-3' ! 31 **N.B.** the actual seq is the  
40 ! reverse complement of the one shown.  
!  
(ON\_LamB3-133) [RC] 5'-ccTcTgAcTgAgT **gcA** cAg -  
!  
45 ! 2 3 4 5 6 7 8 9 10 11 12  
AGt gCT TtA acC caA ccG gcT AGT gtT AGC ggT-  
!  
! 13 14 15  
50 ! tCC ccG g ga cag tca gt -3' ! 2c **N.B.** the actual seq is the  
reverse complement of the one shown.  
!  
(ON\_LamB4-133) [RC] 5'-ccTcTgAcTgAgT **gcA** cAg -  
55 !

! 2 3 4 5 6 7 8 9 10 11 12  
AGt gcT TtA acc caA ccG gcT AGT gtT AGC ggT-  
!  
! 13 14 15  
5 tcC ccG g gg cag agg gt-3' ! 1c **N.B.** the actual seq is the  
reverse complement of the  
one shown.  
!  
(ON\_Lam133PCR) 5'-ccTcTgAcTgAgT **gcA** **cAg** AGt gc-3'

**Table S25** ONs used in Capture of kappa light chains using CJ method and BsmAI

All ONs are written 5' to 3'.

5' READAPTERS (6)	10
ON_20SK15012	gggAggATggAgAcTggTc
ON_20SK15L12	gggAAGATggAgAcTggTc
ON_20SK15A17	gggAgAGTggAgAcTAgTc
ON_20SK15A27	gggTggccTggAgAcTgcgc
ON_20SK15A11	gggTggcTggAgAcTgcgc
ON_20SK15B3	gggAgTcTggAgAcTggTc

Bridges (6)  
 kapbri1012 gggAggATggAgActTggATgtCtTggAcTgtgAcAgAgg  
 kapbri1112 gggAAGATggAgActTggATgtCtTggAcTgtgAcAgAgg  
 kapbri1117 gggAgAGTggAgActTggATgtCtTggAcTgtgAcAgAgg  
 kapbri1A27 gggTggcCTggAgActTggATgtCtTggAcTgtgAcAgAgg  
 kapbri1A11 gggTggcCTggAgActTggATgtCtTggAcTgtgAcAgAgg  
 kapbri1B3 gggAgTCTggAgActTggATgtCtTggAcTgtgAcAgAgg

Primers  
kaPCR11 ccTcTgTcAcAgTgcAcAAgAc  
kapfor 5'-aca ctc tcc cct att gaa oct ctt-3'  
225

**Table 530** PCR program for amplification of kappa DNA

72°C 1 minute  
72°C 7 minutes  
4°C hold

**5 Reagents (100 ul reaction):**

Template	50 ng
10x turbo PCR buffer	1x
turbo Pfu	4U
dNTPs	200 $\mu$ M each
<i>10</i>	300 nM
kaPCrt1	300 nM
kapfor	

1000 999 998 997 996 995 994 993 992 991 990 989 988 987 986 985 984 983 982 981 980 979 978 977 976 975 974 973 972 971 970 969 968 967 966 965 964 963 962 961 960 959 958 957 956 955 954 953 952 951 950 949 948 947 946 945 944 943 942 941 940 939 938 937 936 935 934 933 932 931 930 929 928 927 926 925 924 923 922 921 920 919 918 917 916 915 914 913 912 911 910 909 908 907 906 905 904 903 902 901

Table 610: Stuffer used in VH

	1	TCCGGAGCTT CAGATCTGTT TGCCTTTTG TGGGTGGT CAGATCGGT TAGGGAGATC
	61	GACCGACTGC TTGAGCAAA GCCACGCTTA ACTGGTGA CTACTCTGCA AGCATGGGA TGTATTATGCC
5	121	CAAAACAGTC GTCAGGATCT TAACCTGAGG CTTTTTTAC AAACATTAAC AGCAGGCCACA
	181	TCTGGTTGA CACAGAGCGA TCCGGCTCGT CAGTTGGTAG AGCCAGGCTC TGCCATCCTG
	241	GGCATAATT TGCTTAATGA TGATGGTAAA ACCTGGGAGC AGCCAGGCTC TGCCATCCTG
	301	AACGTTGGC TGACCAAGTAT GTTGAAGGGT ACCGGTAGGG CTGCCTTACCT ATGGCCATT
	361	GATAAGTGT ACAGGCCAG TGGCTACGA ACAACCCAG ACGGCCCCAAC TGGTTGGTGC
	421	AATAAAGTG TTGGAGCAA AATTTTGAT GAGGGGGTGC AGGGAGACAA ATCACCAATC
10	481	CCACAGGGG TTGATCTGTT TGCTGGGAAA CCACAGCAGG AGGTGTGTT GGCTGGCTG
	541	GAAGATACCT GGGAGACTCT TTCCA AACGC TATGGCAATA ATGTGAGTAA CTGGAAAACA
	601	CCTGCAATGG CCTTAACGTT CGGGCAAA ATTTCCTTG GTGTACCGCA GGCGCAGCG
	661	GAAGAAACGC GTCATCAGGC GAGATATCAA AACCGTGGAA CAGAAAACGA TATGATGTT
	721	TTCTACCAA CGACAAAGCGA TCGTCCTGTG CTGCTCTGG ATGTGTGCG ACCCGGTICAG
15	781	AGTGGGTTA TTGCTCCGA TGGAACAGT GATAAGCACT ATGAAGATCA GCTGAAAATG
	841	TACGAAATT TTGGCCGTA GTCGCTCTGG TTAACGAGC AGGATGTGGA GGCGCATAG
	901	GAGTCGTCTA GA





1 HpaI GTTAAC 1 3730  
 1 XbaI TCTAGA 1 3767  
 1  
 5 AfELII CTTAAg 1 3811  
 1 BsmI Ngattc 1 3821  
 1 - - GAATGCN 1 4695  
 1 RsrII CGGwccg 1 3827  
 1 NheI Gctagc 1 4166  
 1 BstEII Ggttnacc 1 4182  
 10 BsmBI CGTCTCNnnn 2 4188 6625  
 1 - - Nnnnnngagacg 1 6673  
 1 ApaI GGGCCC 1 4209  
 1 BanII GRGCYC 3 4209 4492 6319  
 1 Bsp120I Gggccc 1 4209  
 1 PspOMI Gggccc 1 4209  
 1 BseRI NNnnnnnnnnctcc 1 4226  
 1 - - GAGGAGNNNNNNNNNN 1 4957  
 1 ECOXI CCTNNnnnnagg 1 4278  
 1 Pf1FI GACNnnntc 1 4308  
 1 Tth111I GACNnnngtc 1 4308  
 1 Kasi GgcGCC 2 4327 5967  
 1 BstXI CCANNNNNNntgg 1 4415  
 1 NotI GCGGCC 1 4507  
 1 EagI Cggccc 1 4508  
 20 1 BamHI Ggtatcc 1 5169  
 1 BspDI ATcgat 1 5476  
 1 NdeI Cttatg 1 5672  
 1 EcoRI Gaaattc 1 5806  
 1 PsiI TTAtaa 1 6118  
 30 1 DraIII CACNNNgtg 1 6243  
 1 BsaAI YACgtt 1 6246

---

1 gagaaaaagg cCTCGTGata ccgcatttt tataggtaa tgcatgata ataatggttt  
 1 BssSI. (1/2)

35 1 61 cttagACGTc agggtggact ttccggggaa atgtgcgg aaccctatt tttttttttt  
 1 121 tcttaataca ttcaaatatG TATCCcgctca tgagaccata accctgataa atgcttcaat  
 1 181 aatattgaaa aaggaaaggat  
 1 Base # 201 to 1061 = APR gene from pUC119 with some RE sites removed  
 1 BciVI..(1 of 2)

40 1 fM S I Q H F R V A L I P F F A

atg agt att caa cat ttc cgt gtc gcc ctt att ccc ttg ttg

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A	F	C	L	P	V	F	A	H	P	E	T	L	V	K
gca	tgc	ttt	cct	cgtt	ttt	gct	cac	cca	gaa	acc	ctg	qtg	aaa	

	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
E	I	D	L	N	S	G	K	I	I	E	S	F	R	P	
qaa	ctg	qat	ctc	aac	acc	qgt	aaq	atc	ctt	qaq	agt	ttt	cac	ccc	

61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
E	E	R	F	P	M	M	S	T	F	K	V	L	L	C
gaa	gaa	cgt	ttt	cca	atq	atq	acc	act	ttt	aaa	qtq	ctg	cta	tgt

ԵՐԵՎԱՆ

91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
R	R	I	H	Y	S	Q	N	D	L	V	E	Y	S	P
CCGC	cgc	ata	cac	tat	tct	cag	aat	gac	ttg	gtt	gag	TAC	Tca	cca

1100

1106 107 108 109 110 111 112 113 114 115 116 117 118 119 120  
V T E K H L T D G M T V R E L  
gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta

C S A A I T M S D N T A A N L  
tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta

L L T T I G P K E I T A F L  
ttt ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg

H N M G D H V T R L D R W E P

then doth he goe it forth in such a maner as if he  
had bin there to have bin there.

cac aac atg ggg gat cat gta act cgc ctt gat cgt tgg gaa ccc

166 167 168 169 170 171 172 173 174 175 176 177 178 179 180  
 E L N E A I P N D E R D T T M  
 gag ctg aaat gaa gcc ata cca aac qac cgt qac acc atq

```

181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
P V A M A T T L R K L T G E
cct gta GCA ATG gca aca acg ttG CGC Aaa cta tta act ggc gaa
BsrDI... (1/2) FspI... (1/2)

```

241 242 243 244 245 246 247 248 249 250 251 252 253 254 255  
 R G I I A A L G P D G K P S R  
 Cgc ggt atc ATT GCA gca ctg ggg cca gat ggt aag ccc tcc cgt  
 BsrDI:::(2/2)

256 257 258 259 260 261 262 263 264 265 266 267 268 269 270  
 I V Y T T G S Q A T M D E  
 atc gta ttt tac acG ggg agT Cag gca act atg gat gaa  
 AhDI

271 272 273 274 275 276 277 278 279 280 281 282 283 284 285  
 R N R Q I A E I G A S L I K H  
 cga aat aga cag atc gct gag ata ggt gcc tca ctg att aag cat

1056 tgg taa  
W .  
286 287

catatatact ttagattgat ttaaaaacttc atttttaatt taaaaggatc tagtgtaaag  
ctgtcagac caagttaat

65

1

—

10

25

23

43

4

1081

negative group,  $\mu^{\text{neg}} = -\mu$ , where  $\mu$  is the mean of the positive group.

Arg Gln Lys Asp Glu Asn Ser Thr Val Ile Leu

Vlight domains could be cloned in as ApaLI-XbaI fragments.  
VL-CL(kappa) segments can be cloned in as ApaLI-Asci fragments. <-----

5	Ckappa	31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 R G T V A A P S V F I F P P S	cgt gga act gtg gct gca cca tct GTC TTC atc ttc ccg cca tct	BbsI... (1/2)
10		46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 D E Q L K S G T A S V V C L		
15	2404	61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 N N F Y P R E A K V Q W K V D	gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg	
20	2449	76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 N A L Q S G N S Q E S V T E Q	aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat	
25	2494	91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 D S K D S T Y S L S T L T L	aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag	
30	2539	106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 S K A D Y E K H K V Y A C E V	gac agc aag gac acc tac acc ctc acc acc ctc acg CTG	EspI... ...EspI... AccI... (2/2)
35	2584	121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 T H Q G L S S P V T K S F N R	AGC aaa gca gag tac gag aaa cac aaa GTC TAC GCC TGC gaa gtc	AgeI... (1/2)
40	2629	136 137 138 139 140 G E C . .	acc cat cag ggc ctg agt tca CGG GTg aca aag agc ttc aac agg	GG CGGCCaatt AsCI... BssHII.

2701 ctatttcaag gagacagtca ta

PelB::3-23(stuffed)::CH1::III fusion gene

5      1    2    3    4    5    6    7    8    9    10   11   12   13   14   15  
M    K    Y    L    P    T    A    A    G    L    L    L  
2723 atg aaa tac cta ttg cct acg gca gca gct gga ttg tta tta ctc

10

16    17    18    19    20    21    22  
A    A    Q    P    A    M    A  
2768 gcG GCC cag ccG GCC atg gcc

SfiI.....

NgOMIV, (1/2)

NcoI....

FR1 (DP47/V3-23)  
23    24    25    26    27    28    29    30  
E    V    Q    I    L    E    S    G  
gaa|gtt|CAA|TTC|ttt|gag|tct|gg|  
| MfeI |

15

FR1  
31    32    33    34    35    36    37    38    39    40    41    42    43    44    45  
G    G    L    V    Q    P    G    G    S    L    R    L    S    C    A  
2813 |ggc|gg|gtt|gtt|cag|cct|gg|tgc|tct|ttt|cgt|ttt|tgc|gtt|

20

FR1  
46    47    48  
A    S    G  
2858 |gct|TCC|GGG|  
| BspEI |

25

Stuffer for CDR1, FR2, and CDR2  
There are no stop codons in this stuffer.

2867

gtttAGATC Tgtttgcctt  
BglII..  
2887 ttgtgggt ggtggcagtc gggttacgga gatcgaccga ctgttgagg aaagccacg  
cttaactgcT GATCAggcat ggatgttat tcgccaaacc agtcgtcagg atcttaaacct  
BclII..

40

gaggctttt ttacctactc tgcaaggcgc gacatgggt ttgacacaga qcgatccgg  
tcgcgttgc gtgaaaacat taacacgttq ggtggcatc aatttgttta atgatgtgg

3007  
3067

3127 taaaacctgg caggcggccag gctctggccat cctgaaacgtt tggctgacca gtatgttgaa  
3187 gcgtacgta gtggctggc tacctatgcc Atttgtataaag TGGtacgcg ccagtggcta  
XcmI.....

5 3247 cgaacacc accgtggcc caactggatccc aatcccaacat agtgtggag caaaaatttt  
3307 gtatggcg gtggaggag aatggatccgatccgatccgatccgatccgatccgatccgatccg  
3367 gaaaccacag caggaggatc tggtggctgc gctggaaat acctggagaat ctctttccaa  
3427 acgtatgg aataatgtga gtaacttggaa aacacatcgaa atggcttgcgatccgatccgatccg  
3487 aaataatttc ttgtgtgtac cgcaaggccgc aqcgaaagaa ACGGGAGATC aggccggatca  
MluI..

10 3547 tcaaaaacgtt gyaacagaaaa acgatatgtat tgttttctca ccaacgacaa gcatatgtcc  
3607 tggcttggcc tggatgttgg tcgcacccgg tcagatgtgg ttatgtctt ccgtatggaaac  
3667 agtggataag cactatggaa atcagatgtga aatgtggaa aattttggcc gtaaagtgcgt  
PvuII.

15 3727 ctggTAACG aaggaggatg tggaggccgc taaggatgtcg  
HpaI..  
HincII(2/2)

20 3767 |TCT|AGA|gac|aaat|tct|aat|act|lctc|tac|tgc|cag|atgc  
| XbaI |  
-----FR3----->|  
4 5 6 7 8 9 10 11 12 13 14 15 16  
93 94 95 96 97 98 99 100 101 102 103 104 105  
S R D N S K N T L Y L Q M  
|TCT|AGA|gac|aaat|tct|aat|act|lctc|tac|tgc|cag|atgc  
| XbaI |

25 3806 |aac|aag|TTA|AG t ctg agc att CGG TCC G  
|AflII |  
-----FR3----->|  
17 18 19 20  
106 107 108 109  
N S L S I R S G  
|aac|aag|TTA|AG t ctg agc att CGG TCC G  
|AflII |  
RSRII..

30 3834 gg caa cat tct cca aac tga ccagacga cacaacggc  
3872 ttacgttaa tcccgccat gggatggtaa agagggtcg tcttgcgtgg ccttgcgttc  
3932 tcaggtaag gccaatggcggatggc gacacatcg gcaatggaaac aagactgac  
3992 catcaatgg tactatgtcg atgtaaacgg caatatgg tatgtccata ctgtgtgttta  
4052 tccgatgtcg atgtccggc attaccggcgtt ccttgcgttccg gaaaatggaa  
4112 ctggaaaggg ctattggcc ttgaaatgtaa cccttaaagtgtataaccccc ag  
4164 aa GCTAAC ctgcgttcc  
NheI..

40 4182 G|GTC|ACC|  
| BstEII |  
gtc tca agc

.....  
.....  
.....  
.....

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150  
A S T K G P S V F P L A P S S  
gcc tcc acc aag ggc cca tcc gtc tcc ccc ctg gca ccc tcc tcc  
151 152 153 154 155 156 157 158 159 160 161 162 163 164 165  
K S T S G T A A L G C L V K  
aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag  
166 167 168 169 170 171 172 173 174 175 176 177 178 179 180  
D Y F P E P V T V S W N S G A  
gac tac ttc ccc gaa cgg acg gtg acg tgg tgc tgg aac tca ggc gcc  
181 182 183 184 185 186 187 188 189 190 191 192 193 194 195  
L T S G V H T F P A V L Q S S  
ctg acc agg gtc cac acc ttc ccg gct gtc cta cag tcc tca  
196 197 198 199 200 201 202 203 204 205 206 207 208 209 210  
G L Y S L S S V V T V P S S S  
gga ctc tac tcc ctc agc agc gta gtc acc gtc ccc tcc agc agc  
211 212 213 214 215 216 217 218 219 220 221 222 223 224 225  
L G T Q T Y I C N V N H K P S  
ttg ggc acc gag acc tac atc tgc aac gtc aat cac aag ccc agc  
226 227 228 229 230 231 232 233 234 235 236 237 238  
N T K V D K K V E P K S C  
aac acc aag gtc gag aag AAA GTT GAG CCC AAA TCT TGT  
ON-TQHCforw.....

30

Poly His linker  
139 140 141 142 143 144 145 146 147 148 149 150  
A A H H H H H G A A  
GCG GCC GCA cat cat cat cac cat cac ggg gca tag  
NotI.....  
EagI....

151 152 153 154 155 156 157 158 159 160 161 162 163 164 165  
E Q K L I S E E D L N G A A  
gaa caa aaa ctc atc tca gaa gag gat ctg atat ggg gca tag  
Mature III.....>...  
166 167 168 169 170 171 172 173 174 175 176 177 178 179 180

35

40

		T	V	E	S	C	L	A	K	P	H	T	E	N	S	F
	4568	act	gtt	gaa	agt	tgt	tta	gca	aaa	cct	cat	aca	gaa	aat	tca	ttt
5	4633	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
		T	N	V	W	K	D	D	K	T	L	D	R	Y	A	N
		act	aac	gtc	tgg	aaa	gac	aaa	act	tta	aat	cgt	tac	gct	aac	
10	4678	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
		Y	E	G	C	L	W	N	A	T	G	V	V	C	T	
		tat	agg	ggc	tgt	ctg	tgg	AAT	GCT	aca	ggc	gtt	gtg	gtt	tgt	act
		BsmI....														
		211	212	213	214	215	216	217	218	219	220	221	222	223	224	225
15	4723	G	D	E	T	Q	C	Y	G	T	W	V	P	I	G	L
		ggt	gac	gaa	act	cag	tgt	tac	ggt	aca	tgg	gtt	cct	att	ggg	ctt
		226	227	228	229	230	231	232	233	234	235	236	237	238	239	240
20	4768	A	I	P	E	N	E	G	G	S	E	G	G	G	S	
		gct	atc	cct	gaa	aat	gag	ggt	ggt	gac	tct	gag	ggt	ggc	ggt	tct
		241	242	243	244	245	246	247	248	249	250	251	252	253	254	255
		E	G	G	S	E	G	G	G	T	K	P	P	E	Y	
		gag	ggt	ggc	ggt	tct	gag	ggt	ggc	ggt	act	aaa	cct	cct	gag	tac
25	4813	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
		G	D	T	P	I	P	G	Y	T	Y	I	N	P	I	D
		ggt	gat	aca	cct	att	ccg	ggc	tat	act	tat	atc	aac	cct	ctc	gac
30	4858	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285
		G	T	Y	P	P	G	T	E	Q	N	P	A	N	P	N
		ggc	act	tat	ccg	cct	ggt	act	gag	caa	aac	ccc	gct	aat	cct	aat
35	4903	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
		P	S	L	E	S	Q	P	L	N	T	F	M	F	Q	
		cct	tct	ctt	GAG	GAG	tct	cag	cct	ctt	aat	act	ttc	atg	ttt	cag
		BseRI...(2/2)														
40	4948	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315
		N	N	R	F	R	N	R	Q	G	A	L	T	V	Y	T
		aat	aat	agg	tcc	cga	aat	agg	cag	ggt	gca	tta	act	gtt	tat	acg
		316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
		G	T	V	T	Q	G	T	D	P	V	K	T	Y	Y	Q

5038 ggc act gtt actcaa ggc act gac ccc gtt aaa act tat tac cag  
331 332 333 334 335 336 337 338 339 340 341 342 343 344 345  
Y T P V S S K A M Y D A Y W N  
5 5083 tac act cct gta tca tca aaa gcc atg tat gag gct tac tgg aac  
346 347 348 349 350 351 352 353 354 355 356 357 358 359 360  
G K F R D C A F H S G F N E D  
5128 ggt aaa ttc aga gac tgc gct ttc cat tct ggc ttt aat gag GAT  
361 362 363 364 365 366 367 368 369 370 371 372 373 374 375  
P F V C E Y Q G Q S S D L P Q  
5173 CCA ttc gtt tgt gaa tat caa ggc caa tct gac tct GAC CTG Cct caa  
BspMI... (2/2)

5038 ggc act gtt actcaa ggc act gac ccc gtt aaa act tat tac cag

331 332 333 334 335 336 337 338 339 340 341 342 343 344 345

Y T P V S S K A M Y D A Y W N

5 5083 tac act cct gta tca tca aaa gcc atg tat gag gct tac tgg aac

346 347 348 349 350 351 352 353 354 355 356 357 358 359 360

G K F R D C A F H S G F N E D

5128 ggt aaa ttc aga gac tgc gct ttc cat tct ggc ttt aat gag GAT

361 362 363 364 365 366 367 368 369 370 371 372 373 374 375

P F V C E Y Q G Q S S D L P Q

5173 CCA ttc gtt tgt gaa tat caa ggc caa tct gac tct GAC CTG Cct caa

BspMI... (2/2)

376 377 378 379 380 381 382 383 384 385 386 387 388 389 390

P P V N A G G S G G S G G G

5218 cct cct gtc aat gct ggc ggc tct ggt ggt tct ggt ggt ggc

391 392 393 394 395 396 397 398 399 400 401 402 403 404 405

G S E G G S E G G G S E G G

5263 ggc tct gag ggt ggc ggc tct gag ggt ggc ggt tct gag ggt ggc

406 407 408 409 410 411 412 413 414 415 416 417 418 419 420

G S E G G S G G S G S G D

5308 ggc tct gag ggt ggc ggt tcc ggt ggc ggc tcc ggt tcc ggt gat

421 422 423 424 425 426 427 428 429 430 431 432 433 434 435

F D Y E K M A N A N K G A M T

5353 ttt gat tat gaa aaa atg gca aac gct aat aag ggg gct atg acc

436 437 438 439 440 441 442 443 444 445 446 447 448 449 450

E N A D E N A L Q S D A K G K

5398 gaa aat gcc gat gaa aac ggc cta cag tct gac gct aaa ggc aaa

451 452 453 454 455 456 457 458 459 460 461 462 463 464 465

L D S V A T D Y G A A I D G F

5443 ctt gat tct gtc gct act gat tac gtc gct ggt gct gtc ATC GAT ggt ttc

BspDI...

466 467 468 469 470 471 472 473 474 475 476 477 478 479 480

I G D V S G L A N G N G A T G

40

5488 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt  
 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495  
 D F A G S N S Q M A Q V G D G  
 5 5533 gat ttt gct ggc tct aat tcc caa atg gct caa gtc gac ggt  
 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510  
 D N S P L M N N F R Q Y I P S  
 5578 gat aat tca cct tta atg aat aat ttc cgt caa tat tta cct tct  
 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525  
 L P Q S V E C R P Y V F G A G  
 5623 ttg cct cag tcg gtt gaa tgt cgc cct tat gtc ttt ggc gct ggt  
 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540  
 K P Y E F S I D C D K I N L F  
 15 5668 aaa CCA TAT Gaa ttt tct att gat tgt gac aat ata aac tta trc  
 NdeI...  
 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555  
 R G V F A P L L Y V A T F M Y  
 20 5713 cgt ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat  
 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570  
 V F S T F A N I L R N K E S  
 25 5758 gta ttt tcg acg ttt gct aac ata ctg cgt aat aag gag tct taa  
 571  
 30 5803 taa GAATTC  
 EcoRI.  
 5812 actggccgt cggtttacaa cgtcgtgact gggaaaaccg tggcggttacc caacttaatc  
 5871 gccttgcaggc acatccccct ttcgcggctt ggcgttaatg cgaayaggcc cgcacCGATC  
 PvUI..  
 35 5931 Gcccttccca acagtTGCGC Agcctgaatg gcgaatGGG CCTgatGGG tattttctcc  
 ...PvUI... (3/3) FspI... (2/2) Kesi... (2/2)  
 5991 ttacgcatct gtgcgttatt tcacaccgca tataatgt aaacgttaat atttttgtaa  
 6051 aatcgcgtt aaattttgt taaatcgtt cattttttaa ccaataggcc gaaatcgcca  
 6111 aaatccccTA TAAatccaaa gaatagccg agatagggtt gatgtgtttt ccagtttggaa  
 40 PsiI...  
 acaaggatcc actttaaag aacgtggact ccaacgtcaa aggccaaaaa accctctatc  
 6171 agggcgatgg cccACTacGT Gaaccatcac ccaaattcaag ttttttttttggg tcgagggtgcc  
 6231 DraIII...  
 !

6291 gtaaaggcact aaatcgaaac cctaaaggga gcccccatt tagagcttgc aaggaaaaGC		NgOMI	
6451	CGCCgaacgt	ggcgagaaaag	aaggaaaaaa
	caagtgttag	ggcacgt	ccacacccgc
6411	..NgoMIV. (2/2)	cgtgtaacca	cgcgttaat
6471	aggcgctgt	tttgacccat	ggatgttcgt
6531	catagttaa	ccatggccaa	caccggccaa
6591	tgcccccggc	atccgcttac	agacaaggctg
6651	ggttttcacc	tgaccgttgc	cggggactgc
			aaacgcgcgg

Table 630: Oligonucleotides used to clone CDR1/2 diversity

All sequences are 5' to 3'.

5    1) ON\_CD1Bsp, 30 bases

A	C	C	T	C	A	C	T	G	G	C	T	T	C	C	g	g	A
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

T	T	C	A	C	T	T	T	C	T	C	T	C	T	C	T	C	T
19	20	21	22	23	24	25	26	27	28	29	30						

2) ON\_Br12, 42 bases

A	g	A	A	A	C	C	C	A	C	T	C	C	A	A	A	C	C
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

T	T	T	A	C	C	A	G	G	A	G	C	T	T	g	g	C	g
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36

A	A	C	C	C	A												
37	38	39	40	41	42												

3) ON\_CD2Xba, 51 bases

g	g	A	A	g	g	C	A	g	T	g	A	T	C	T	A	g	A
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

g	A	T	A	g	T	g	A	A	g	C	g	A	C	C	T	T	T
30	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35

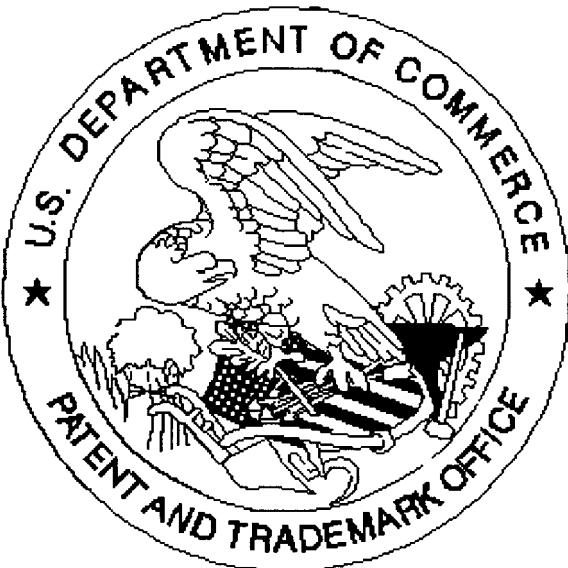
A	A	C	g	g	A	g	T	C	A	g	C	A	T	A			
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51			

35    4) ON\_BotXba, 23 bases

g	g	A	A	g	g	c	A	g	T	g	A	T	C	T	A	g	A
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
g	A	T	A	g													
5	19	20	21	22	23												

## 10 End Tables

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Specification starts from page 2.

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